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Journal of the Fisheries Research Board of Canada

Variation of Organochlorine Residue Levels with Age in Gulf of St. Lawrence Harp Seals (*Pagophilus groenlandicus*)

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Addison, R. F., S. R. Kerr, J. Dale, and D. E. Sergeant. 1973. Variation of organochlorine residue levels with age in Gulf of St. Lawrence harp seals (*Pagophilus groenlandicus*). J. Fish. Res. Board Can. 30: 595–600.

Blubber samples from 18 harp seals taken near the Saguenay River in the Gulf of St. Lawrence were analysed for DDT metabolites, PCBs, and dieldrin. The concentration of total DDT and metabolites ($\sum$DDT) ranged from 3.1 to 22.6 ppm, PCBs (as Aroclor® 1254) from 2 to 22 ppm, dieldrin from 0.1 to 0.3 ppm of blubber. Rank correlation of residue level and age indicated that significant portions of the $\sum$DDT and PCB variance could be assigned to increased residue levels with age. Within the $\sum$DDT group, DDE alone showed a strong proportional increase in concentration with age. Dieldrin concentration was not appreciably correlated with age. Among factors other than age, changes in blubber thickness did not account for any significant amount of the variance in blubber residue concentration. Examination of other published data suggested that parturition and/or lactation may cause reductions in blubber residue concentrations.

Addison, R. F., S. R. Kerr, J. Dale, and D. E. Sergeant. 1973. Variation of organochlorine residue levels with age in Gulf of St. Lawrence harp seals (*Pagophilus groenlandicus*). J. Fish. Res. Board Can. 30: 595–600.

Les auteurs ont dosé les métabolites du DDT, les PCB et la dieldrine dans des échantillons de couche de graisse de 18 phoques du Groenland capturés près de l'embouchure du Saguenay, dans le golfe Saint-Laurent. La teneur en DDT total et en métabolites ($\sum$DDT) varie de 3.1 à 22.6 ppm, celle des PCB (sous forme d'Aroclor® 1254) de 2 à 22 ppm, celle de la dieldrine de 0.1 à 0.3 ppm de graisse. La corrélation des rangs entre le niveau des résidus et l'âge démontre que des portions significatives de la variance de $\sum$DDT et de PCB sont attribuables à l'augmentation des niveaux de résidus en fonction de l'âge. Parmi le groupe des $\sum$DDT, seul

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le DDE accuse une forte augmentation de concentration proportionellement à l'âge. La teneur en dieldrine est relativement indépendante de l'âge. Parmi les facteurs autres que l'âge, les variations d'épaisseur de la couche de graisse ne contribuent pas à augmenter de façon appréciable la variance de la concentration de résidus dans la couche de graisse. Un examen des autres données publiées suggère que la parturition ou la lactation, ou les deux, peuvent réduire la teneur en résidus dans la couche de graisse.

Received October 20, 1972

ORGANOCHLORINE pesticide and polychlorinated biphenyl (PCB) residues have been reported to occur in seals from western Europe, eastern and western North America, and the Antarctic (Koeman and van Genderen 1966; Holden and Marsden 1967; Holden 1970; Anas and Wilson 1970a, b; Shaw 1971). Wide variations in residue level have been observed, often in samples of the same species taken from the same locality (Holden and Marsden 1967; Holden 1970; Anas and Wilson 1970a, b); pups and fetuses, for example, generally contained lower residue levels than did adult animals. The data imply that variable residue levels in seals could be due in part to age differences or causes associated with age. The study described here was undertaken to investigate the relationship of organochlorine residue levels to age in harp seals (*Pagophilus groenlandicus*) from the Gulf of St. Lawrence.

Materials and Methods

Eighteen seals were killed in the Gulf of St. Lawrence in the Tadoussac–Escoumins region (northeast of the mouth of the Saguenay River) in January, 1971.

Blubber samples were taken from the mid-dorsal region, and were frozen and shipped to Dartmouth in glass jars for analysis. Data for the sex, age, and blubber thickness of each specimen are shown in Table 1. Age was determined from the number of layers in the dentine of one of the lower canine teeth examined in the cross section (Laws 1962).

Lipid was extracted from 10 g of blubber by homogenizing in a stainless steel blender with approximately 100 ml of redistilled *n*-hexane, and was estimated gravimetrically after removal of solvent by rotary evaporation. Small samples (about 350 mg) of recovered lipid were taken for pesticide analysis, substantially as described by Addison et al. (1972), with the following modifications. Gas–liquid chromatographic analysis was carried out with a Packard 7400 instrument fitted with a ^{63}Ni electron capture detector. PCBs were estimated from the average peak height ratios of peaks A, B, and C, (Fig. 1) after dehydrochlorination, to those in standard Aroclor® 1254. All analyses were carried out by the same operator using the same batch of standards throughout.

Results and Discussion

Analytical data are shown in Table 1. Lipid accounted for 86–98.6% of whole blubber, and residue data in Table 1 are expressed as ppm of whole blubber. Total DDT ($\sum$DDT = DDE + DDD + DDT) ranged from 3.1 to 22.6 ppm, PCBs from 2 to 22 ppm, and dieldrin (13 samples only) from 0.1 to 0.3 ppm of whole blubber. In the DDT group, only *p,p'* isomers were detected; no *o,p'* isomers were present in sufficient quantity to be estimated in the presence of PCBs, either before or after dehydrochlorination. On the basis of duplicate analyses performed in this laboratory on similar samples, we estimate the data presented in Table 1 for major components (DDE and DDT) to be accurate within $\pm 5\%$ over the range of concentrations reported here. Precision is much reduced for minor components (DDD and dieldrin). The measurement of PCBs, which involves estimation of various components of largely unknown structure, whose proportions may vary from sample to sample, and which differ from those in standard materials, is necessarily less accurate. These residue levels are in the same range as those reported for grey (*Halichoerus grypus*) and harp seals from Cabot Strait, but are higher than levels reported for grey and harp seals from the Magdalen Islands (Holden and Marsden 1967). Our data are slightly lower than residues determined in grey seals from Sable Island and Basque Island (Holden 1970), and are well below the high levels reported by Gaskin et al. (1971) for Bay of Fundy porpoises. The absence of any clear geographic pattern in organochlorine residue levels of marine mammals in Atlantic Canada may be real, but it is equally possible that geographic influence could be confounded with other factors in the available data.

Inspection of Table 1 suggests that part of the observed variability among our determinations is due to a tendency for residue levels to increase with age. The apparent tendency was evaluated by calculating the Kendall rank correlation coefficient (Siegel 1956) as a measure of the association between age and observed concentrations of each contaminant. Estimated values of Kendall's τ are set out together with the corresponding probability levels in Table 2. The coefficients determined for PCBs, DDE, and $\sum$DDT are significant at levels of $P < .01$, but DDD, DDT, and dieldrin are not shown to be significantly related to age by this test.

TABLE 1. Age, size, sex, and blubber residue concentrations of Gulf of St. Lawrence harp seals.

Age (years)	Girth (*cm*)	Length (*cm*)	Sex	Wt (*kg*)	Blubber thickness (*cm*)	Blubber lipid (%)	Residue level in blubber (*ppm*)					
							DDE	DDD	DDT	∑DDT	PCB	Dieldrin
1	99	123	F	51.7	4.0	94.0	1.6	0.4	1.6	3.6	3	0.1
1	92	127	M	49	4.5	90.3	2.8	0.6	2.3	5.7	3	0.1
2	104	127	M	66.2	6.0	87.5	2.4	0.5	2.0	4.9	3	0.1
3	101	135	F	62.1	3.3	95.6	7.1	0.7	4.3	12.1	15	0.3
3	98	139	F	59	4.0	87.5	3.0	0.3	1.7	5.0	6	0.1
3	102	146	M	62.6	3.7	91.3	2.9	0.8	2.7	6.4	6	NA[a]
3	114	147	M	73.9	4.0	87.3	2.1	0.0	1.0	3.1	6	0.1
3	108	152	M	74.4	4.0	97.0	1.7	0.4	1.3	3.4	2	NA
3	113	138	M	70.3	4.2	91.0	3.1	1.1	3.1	7.3	3	NA
5	111	146	M	77.1	3.5	90.8	5.5	1.2	3.8	10.5	7	NA
6	124	159	F	105.7	4.9	89.1	3.1	0.3	1.5	4.9	3	NA
6	123	167	M	113	5.2	86.0	4.1	0.6	2.2	6.9	10	0.1
8	126	161	M	112.9	4.5	98.6	2.9	0.6	2.0	5.5	6	0.3
9	122	168	M	105.7	4.1	92.8	4.4	0.7	3.4	8.5	13	0.3
11	124	168	M	107.5	4.5	92.1	14.5	1.4	6.7	22.6	22	0.3
13	127	163	M	113.9	5.7	89.2	15.3	0.6	5.6	21.5	21	0.1
13	121	165	M	105.7	4.5	90.7	16.4	1.5	4.1	22.0	9	0.2
18	142	177	M	148.3	7.5	87.6	10.9	0.6	3.0	14.5	9	0.1

[a]NA = Sample not analysed.

Comparison of the coefficients calculated for the three components of ΣDDT indicates that the relationship of DDE with age accounts for the major portion of the overall correlation. This conclusion would be expected if DDE were the principal storage metabolite of the DDT group in seals, as it is in most mammals so far studied (O'Brien 1967; Menzie 1969). Relatively low concentrations of DDD may have contributed to decreased analytical accuracy and weakened the strength of its correlation with age, but DDD is thought to be an intermediate in the degradation of DDT in some mammals (O'Brien 1967; Menzie 1969) and may exist at a relatively low steady state concentration in the animal. The correlation between PCB concentration and age is similar to that determined for ΣDDT, but calculations with the relative proportions of the three PCB peaks used for quantitation (Fig. 1) do not demonstrate any changes with age. The absence of a significant correlation between dieldrin concentration and age may be due to decreased analytical accuracy when residues are in low concentrations, but low concentrations might themselves reflect relatively rapid rates of residue clearance leading to steady state concentrations.

Our conclusion that organochlorine residues may accumulate in seals over long periods of time is predictable, in view of what is known of the persistence and stability of these compounds. So far as we are aware, however, the phenomenon has not previously been demonstrated explicitly for

TABLE 2. Values of Kendall rank correlations (τ) and the probabilities of their occurrence (P) for residue levels in blubber (*ppm*) with age (yrs). Data taken from Table 1.

Residue	τ	P
ΣDDT	0.38	<0.01
DDE	0.51	<0.01
DDD	0.17	0.15
DDT	0.24	0.08
PCB	0.44	<0.01
Dieldrin[a]	0.17	0.20
DDE as % ΣDDT	0.47	<0.01
DDD as % ΣDDT	−0.21	0.11
DDT as % ΣDDT	−0.49	<0.01

[a]only 13 samples analysed.

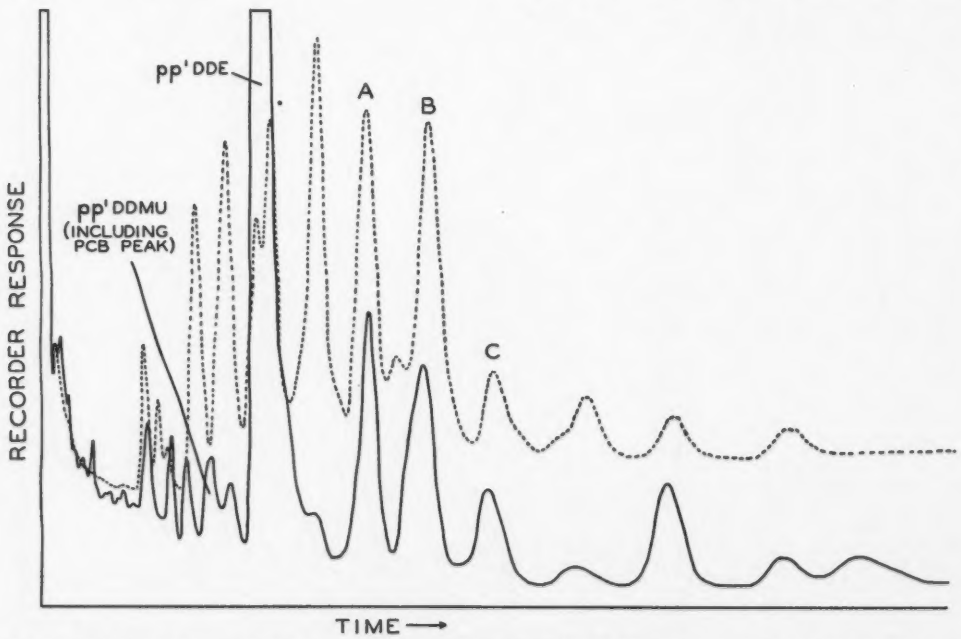

FIG. 1. Gas–liquid chromatograms of seal blubber residues cleaned up on Florisil and subjected to dehydrochlorination (solid line) and Aroclor® 1254 (2.5 ng) (broken line), run on DC 200 as described in text.

seals, although similar relationships have been observed for various fish species (Anderson and Fenderson 1970; Bache et al. 1972; Youngs et al. 1972).

One factor complicating our discussion of residue level variation with age is that the data describe *concentrations* of residues in blubber, rather than the quantity of residue contained by the organism. By this reasoning, lean animals would exhibit higher residue concentrations than fatter ones containing the same total residue burden. To investigate this possibility, we used girth, length, and blubber thickness data (Table 1) to calculate measures of blubber volume relative to total body volume. Estimated variation in relative blubber volume does not account for our observed correlations between residue concentration and age. Calculation of Kendall partial rank correlation coefficients of the age (x) with residue level (y) and blubber thickness (z) showed that $\tau_{xy.z}$ was not substantially increased over τ_{xy}; i.e. variation of blubber thickness did not contribute appreciably to the overall variability of the data.

The sex and reproductive state of test organisms could also influence residue concentration. The results of Holden (1970) imply an increase with age in organochlorine residue level in Sable Island Grey seal blubber (Table 3, Holden 1970) but age determinations were not available to him. We have reexamined his data and calculated correlations of residue level with age from determinations now available. Relationships similar to, but much weaker than those in harp seals may exist (Table 3), but are not significant by the conservative satistical procedure we used. The greater variability of Holden's specimens may be due to a high proportion of lactating females and nursing pups in the sample (13 out of 16 specimens). Parturition and lactation could reduce the residue burden of the mother; some indication of this can be obtained by comparison of residue levels of the same mean age. The mean ages of lactating and nonlactating seals 10 years old or more were similar (16.2 and 16.6, respectively) but mean residue levels differed widely (14.7 and 49.1 ppm, respectively). Similar differences, all suggesting reduced residue levels during lactation, can be calculated for other contaminants in these samples. None of the differences was significant by statistical test, due to the wide variance shown by the few nonlactating seals. Nevertheless, the differences suggest a source for some of the variance in Holden's data, and, more important, imply that reproductive state is another variable to be considered in assessing observed contaminant concentration in seals. Gaskin et al. (1971) have reached similar conclusions about organochlorine residues in harbour porpoises *Phocoena phocoena*.

TABLE 3. Values for Kendall rank correlation coefficients (τ) and the probabilities of their occurrence (P) for residue levels with age. Data from Holden 1970, as described in text.

Residue	τ	P
ΣDDT	0.18	0.17
DDE	0.16	0.19
DDT	0.19	0.15

The only data available in the literature for which similar relationships with age can be calculated are those of Anas and Wilson (1970), describing residue concentration in Pribilof Islands fur seals (*Callorhinus ursinus*). These data do not indicate any significant change in residue levels with age (correlation calculated by us). However, their analyses were of residue concentrations in liver tissue, which may not necessarily vary with age in the same way as blubber concentration. Moreover, all of the 23 nonpregnant seals analysed were 10 years old or less, and 20 of these were 4 years old or less. It is perhaps important to note that the significant correlation of blubber residue levels with age observed in our own data depends on the inclusion of results from animals more than 10 years old.

Many additional factors undoubtedly affect observed residue concentrations. In our samples, variation in space and time in the availability of residues to the biota of the Gulf of St. Lawrence could have been important. Major insect control programs provided varying levels of DDT input to the adjacent watershed during the lifetimes of the seals we analysed (eg. Kerswill 1967); in particular, the oldest and youngest specimens may have been subjected to lower rates of exposure over their lifetimes than the remainder of the sample. Moreover, in the absence of more extensive data we cannot distinguish between nonequilibrium residue accumulations determined by total length of exposure to contaminants, and different equilibria such as might be exhibited by seals undergoing qualitative changes in contaminated diet with age.

Taken together, various parameters seem capable of substantial effects on observed residue levels and clearly must be considered in the design of future sampling programs. For this reason the correlation of residue level with age reported here should be regarded as a description of our data, and not as a necessary consequence of the processes underlying residue accumulation. Indeed, consider-

ation of the dynamics of residue formation suggests that the salient processes may be essentially non-linear (Kerr and Vass 1973).

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Sea Lamprey (*Petromyzon marinus*) Predation on Freshwater Teleosts

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Farmer, G. J., and F. W. H. Beamish. 1973. Sea lamprey (*Petromyzon marinus*) predation on freshwater teleosts. J. Fish. Res. Board Can. 30: 601–605.

The incidence of attack by the sea lamprey (*Petromyzon marinus*) was studied on freshwater teleosts of similar and different size. Splake (*Salvelinus namaycush* × *Salvelinus fontinalis*), carp (*Cyprinus carpio*), and white suckers (*Catostomus commersoni*) were attacked a significantly greater number of times than lake whitefish (*Coregonus clupeaformis*), burbot (*Lota lota*), shorthead redhorse suckers (*Moxostoma macrolepidotum*), and brown bullheads (*Ictalurus nebulosus*). Lampreys rarely preyed upon walleye (*Stizostedion vitreum vitreum*). Most attacks occurred between the head and caudal peduncle below the lateral line and particularly in the region behind the pectoral fins. Lampreys attacked the largest individuals of any species more frequently than the smaller representatives. The presence of lampreys on a fish did not further the incidence of attack on that fish. None of the species of fish appeared to avoid lamprey attacks, nor did they avoid fish on which lampreys were attached. Only rarely were fish observed to dislodge lampreys.

Farmer, G. J., and F. W. H. Beamish. 1973. Sea lamprey (*Petromyzon marinus*) predation on freshwater teleosts. J. Fish. Res. Board Can. 30: 601–605.

Nous avons déterminé l'occurrence d'attaques par la grande lamproie marine (*Petromyzon marinus*) sur des téléostéens d'eau douce de même taille et de tailles différentes. Les attaques sont significativement plus fréquentes sur la truite moulac (*Salvelinus namaycush* x *Salvelinus fontinalis*), la carpe (*Cyrpinus carpio*), et le catostome noir commun (*Catostomus commersoni*) que sur le grand corégone (*Coregonus clupeaformis*), la lotte (*Lota lota*), le moxostome à cochon (*Moxostoma macrolepidotum*), et la barbotte brune (*Ictalurus nebulosus*). La lamproie n'attaque que rarement le doré jaune (*Stizostedion vitreum vitreum*). La plupart des attaques se localisent entre la tête et le pédoncule caudal, sous la ligne latérale, plus particulièrement dans la région située derrière les nageoires pectorales. Les grands individus, quelle que soit l'espèce, sont attaqués plus fréquemment que les petits. La présence de lamproies sur un poisson ne contribue pas à augmenter la fréquence des attaques sur ce même poisson. Parmi les espèces étudiées, aucune ne semble éviter les attaques de lamproies, ou encore éviter les poissons sur lesquels il y a déjà des lamproies. Ce n'est que rarement qu'un poisson déloge une lamproie.

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Most fishes in the Great Lakes are susceptable to attack by sea lampreys but some have suffered greater predation (B. R. Smith 1972). Habitat and size of host species appear to influence the intensity of predation by sea lampreys (S. H. Smith 1972). Thus, in the upper Great Lakes the large deepwater species, lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*), and the largest species of ciscoes occupying deepwater, *Leucichthys*[1] *nigripinnis* and *L.*[1] *johannae*, were first to decline (S. H. Smith 1972). This was followed by a reduction in the numbers of medium sized deepwater species and larger species from the shallower depths.

In the present study host species and size preferences of the sea lamprey (*Petromyzon marinus*) were examined for a number of teleosts in a large tank of fixed depth and temperature.

Materials and Methods

During October of 1969 and 1970, sea lampreys in the final stages of metamorphosis (Beamish and Potter 1972) were collected by electrofishing from Shelter Valley Creek, a tributary of Lake Ontario.

[1]Author's preference. "*Coregonus*" in A.F.S. listing.

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Lampreys were held at 10±1 C in 2000-liter circular tanks supplied with aerated well water and were subjected to a photoperiod of 16 hr. They were allowed to feed ad libitum on white suckers (*Catostomus commersoni*) for at least 2 months before being used for experimentation.

In the species preference experiment one individual of each of the eight species used was placed in a 40,750-liter tank (10±1 C) for 24 hr before the same number of lampreys was introduced. Special effort was made to select fish of similar length and weight. During the subsequent 24 hr, observations were made hourly except during the 8 hr of darkness. The number and site of lamprey attacks on the various species and the behavior of the experimental animals was recorded. Attacks where lampreys remained attached to their hosts for the duration of an experiment were distinguished from those in which lampreys detached before the end of an experiment. At the end of 24 hr the teleosts and lampreys were removed and the teleosts examined for marks or wounds to ascertain if any unrecorded attacks had taken place during darkness. In total, 120 teleosts and a similar number of lampreys were used in the 15 experiments, an individual being used only once. Food was withheld from all teleosts for at least 1 week before being exposed to lampreys. Lampreys were allowed to feed on white suckers until just prior to an experiment.

The teleosts used were splake (*S. namaycush* × *S. fontinalis*), lake whitefish (*Coregonus clupeaformis*), white suckers, shorthead redhorse suckers (*Moxostoma macrolepidotum*), carp (*Cyprinus carpio*), brown bullheads (*Ictalurus nebulosus*), burbot, and walleye (*Stizostedion vitreum vitreum*). Fish, with the exception of splake and whitefish, were captured by trapnet during October and November 1970 and held in outdoor ponds where they were allowed to feed on common shiners (*Notropis cornutus*) or aquatic vegetation (*Chara* sp., *Spirogyra* sp., and *Potamogeton* sp.). This diet was supplemented with commercial fish pellets once weekly. Two weeks prior to the beginning of the experiments (February 1971), fish were moved to 2000-liter indoor tanks and deprived of food. Whitefish, caught during May 1970, were held in indoor 2000-liter tanks and allowed to feed on emerald shiners (*N. atherinoides*). Splake were obtained from a hatchery 1 week prior to the beginning of the experiment.

The relation between number of lamprey attacks and host size was determined for four individuals of a given species each corresponding to a different weight-class (March 1971). Fish of a given species were placed in a 21,000-liter tank (10±1 C) and allowed a day before an equal number of lampreys were added for the same period. Observations were made over a 24-hr period. Five experiments were made for each of carp, white suckers, and splake. In total, 20 different teleosts and lampreys were used for each species. Six experiments were made for three weight-classes of lake trout (*S. namaycush*) in which 18 different teleosts and lampreys were involved.

The relative numbers of single and multiple attacks on white suckers of similar size were examined to determine if lampreys were attracted to fish that had been attacked (March 1971). Eight white suckers of similar size were placed in the 40,750-liter tank (10±1 C) with an equal number of lampreys for 24 hr and the distribution of attacks was recorded. A total of 25 experiments were made with 200 suckers and a similar number of lampreys, each being used only once.

Multiple Attacks

Single attacks on white suckers of similar size were most common, numbering 92. The number of fish receiving two, three, and four attacks was 51, 18, and 5, respectively. No fish received more than four lamprey attacks. A hypothetical binomial distribution (Fig. 1) describing the relation between single and multiple attacks was calculated from the observed sample mean (Snedecor 1959). That the attachment of one or more lampreys on a given host did not influence additional attacks on that fish was demonstrated by the similarity between the hypothetical and observed distributions that were not significantly different at $P < 0.05$. Had lampreys been attracted to suckers with attached lampreys, the incidence of multiple attacks would have been greater and of single attacks less.

Size Preference

Sea lampreys given a choice of fish of different weight, but of the same species, selected the largest individuals most frequently (Fig. 2A). For each species the relation between number of lamprey

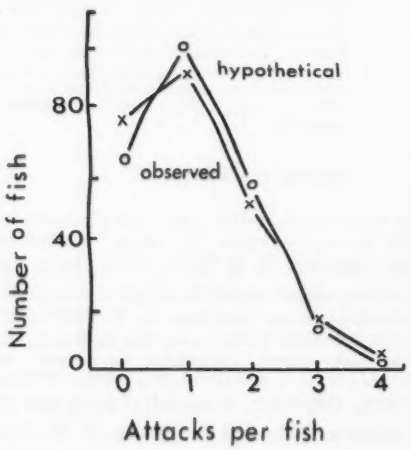

FIG. 1. Observed and hypothetical distributions of single and multiple attacks on white suckers (*Catostomus commersoni*) of similar size. Mean weight of the lampreys was 15 g.

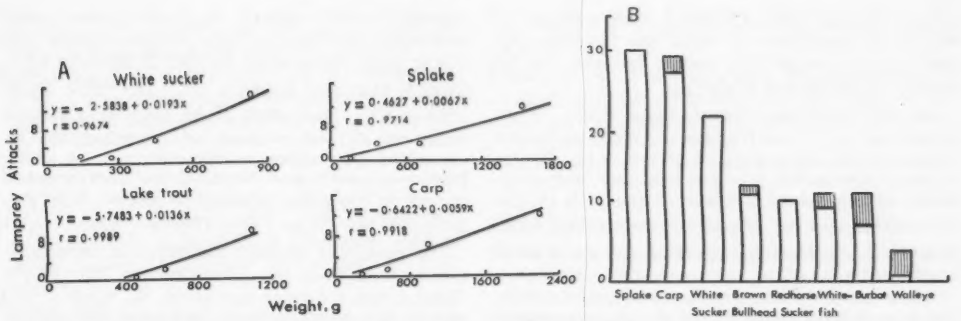

FIG. 2. A, Relation between total number of lamprey attacks on various weight-classes of different teleosts. Mean weight of the lampreys was 25 g; B, Number of lamprey attacks on different teleosts. Shaded areas of histograms represent the number of lamprey detachments. Mean weight of the lampreys was 10 g.

attacks and weight of the teleosts can be described by a linear regression the coefficient of which is dependent on the weight range used. Thus, the coefficients were found to decrease as the weight ranges used for the various species increased.

The higher incidence of attack on the larger individuals parallels field results in which scarring rate has been correlated with size of fish for splake (Berst and Spangler 1970), white suckers (Hall and Elliott 1954; Coble 1967), rainbow trout (*Salmo gairdneri*) (Berst and Wainio 1967), and lake trout (Wigley 1959; Budd et al. 1969).

Species Preference

Splake and carp were most frequently attacked by sea lampreys with 30 and 27 attachments, respectively, followed by white suckers with 22 (Fig. 2B). Significant differences ($P < 0.05$) in number of attacks on the three species were not demonstrable using Duncan's New Multiple Range test (Li 1964). Attacks on brown bullheads, shorthead redhorse suckers, lake whitefish, and burbot were about equal in numbers and less than half those on splake, carp, and white suckers. The numbers of lamprey attacks among these species were not different ($P < 0.05$) but were significantly less than those on splake, carp, and white suckers. The least attacked fish was walleye on which only one lamprey remained attached for the duration of an experiment. Walleye were attacked on three other occasions but lampreys always detached within a short period. Lamprey detachments were generally restricted to those species receiving the fewest number of attacks. Numbers of lamprey attacks used in the statistical analysis did not include lamprey detachments.

The mean lengths and weights of fish used were:

	Length (*cm*)	Weight (*g*)
Burbot	49.6	680
Walleye	42.9	828
White sucker	42.3	870
Lake whitefish	40.7	547
Shorthead redhorse sucker	40.6	819
Splake	40.3	764
Carp	37.1	708
Brown bullhead	30.2	414

Bullheads were the only species significantly less than the others in both length and weight ($P < 0.05$) using Duncan's New Multiple Range test. Possibly bullheads would have received more lamprey attacks had they been of equal size.

The disproportionate number of attacks on the various species cannot apparently be explained by behavioral differences among hosts. In no instance did any of the species subjected to predation attempt to avoid lamprey attacks, few of which were unsuccessful.

Most of the teleosts concentrated near the center of the tank near the bottom, although burbot and, to a lesser extent, walleye were found nearer the walls. None of the teleosts avoided lampreys swimming nearby and those that had lamprey feeding on them were not avoided by the other fish. When attacked, fish usually tried to dislodge the lamprey by scraping against the bottom of the tank but this was invariably unsuccessful and of

short duration. Splake, burbot, and walleye occasionally attempted to ingest sea lampreys but they usually escaped by passing between the gill arches and operculum of the fish.

Wounds occurring during the duration of an experiment were most frequent in attacks on brown bullheads, the only scaleless teleost used. Of the 11 sea lampreys that attacked bullheads, nine penetrated into the muscle tissue of the host (82%). In contrast, seven of 27 attacks on carp (26%) and four of 22 attacks (18%) on white suckers resulted in wounding.

The incidence of attack on male and female teleosts was examined for splake. Of the 30 attacks on splake, 15 were made on males and 15 on females.

The most frequently attacked site on the teleosts was below the lateral line and between the head and caudal peduncle (69%). Of these, almost half were behind the pectoral fins. Seventeen percent of the attacks were above the lateral line and between the head and caudal peduncle, and 10% on the head. Attacks rarely occurred on the caudal peduncle and fins.

The site of lamprey attack on the various teleosts is in accord with results of field studies. For example, Wigley (1959) found that the greatest number of scars on lake trout from Lake Cayuga were below the lateral line and between the head and caudal peduncle, 45% of all scars being near the pectoral fins. Similarly, Berst and Wainio (1967) reported that 89% of lamprey scars observed on rainbow trout from Lake Huron were below the lateral line and between the head and caudal peduncle. Fifty-one percent of all scars were near the pectoral fins. A restriction of most feeding to ventral areas, particularly at the site behind the pectoral fins, may be explained by the thinner muscular layer and greater distribution of blood in this area. In addition, it may be more difficult for teleosts to dislodge lampreys attached ventrally, particularly when they are protected by the pectoral or pelvic fins.

In the sea lamprey, Kleerekoper (1969) stressed the importance of olfaction in the procurement of prey. He identified an amine (isoleucine methyl ester) excreted by teleosts that was effective in eliciting locomotor responses in lampreys. Kleerekoper and Morgensen (1963) found this amine in variable concentrations in the body odors of different species, which may explain the greater incidence of attack on certain species in this study.

It is of interest to compare the pattern of lamprey predation in the experimental tank with that in the Great Lakes. Splake and white suckers, attacked frequently under experimental conditions, are also hosts for lampreys in the Great Lakes. For example, in South Bay of Georgian Bay, Berst and Spangler (1970) reported that 8% of the splake examined bore one or more lamprey marks, and Coble (1967) found scars on 10% of the large white suckers examined between 1947 and 1953. From 1953 to 1961, up to 40% of the large white suckers examined showed evidence of predation, the increase being coincident with the destruction of the lake trout population. Walleye, the least attacked species in the study, appears to receive little predation in the Great Lakes (Ryder 1968).

The pattern of lamprey preference in the experimental tank does not correspond to that in the Great Lakes for some species. B. R. Smith (1972) reports that populations of cold-water fish such as lake trout, burbot, rainbow trout, and whitefish declined abruptly in lakes Huron and Michigan because of lamprey predation. However, the incidence of attack on burbot and whitefish was low in this study and, in a preliminary experiment with hatchery splake and rainbow trout and "wild" lake trout of similar size, splake were attacked five times as frequently as either rainbow or lake trout. Thus, while lampreys in a laboratory situation discriminate between host species, the pattern of preference is not always the same as in the Great Lakes. This would suggest that the ecological distribution of host species and their relative size are of particular importance in determining lamprey predation. In this context S. H. Smith (1972) suggests that the cool deeper areas of the Great Lakes are preferred by lampreys.

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Determination of Age and Growth of Populations of the White Sucker (*Catostomus commersoni*) Exhibiting a Wide Range in Size at Maturity

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BEAMISH, R. J. 1973. Determination of age and growth of populations of the white sucker (*Catostomus commersoni*) exhibiting a wide range in size at maturity. J. Fish. Res. Board Can. 30: 607–616.

The use of the scale method to determine age of mature white suckers should be avoided. Sections of pectoral fin rays can provide accurate age determinations for most populations once the position of the first annulus has been identified. The age of immature white suckers can be estimated by both methods. It is suggested that, for identification of the first fin ray annulus and facility in the determination of the age of immature white suckers, both pectoral fins and scales should be sampled in all age and growth studies of this species.

White suckers (*Catostomus commersoni*) from populations examined in this study attained ages of up to 17 years although little or no growth occurred in the latter years. Considerable variation in size at sexual maturity was found throughout the range for this species and, thus, the validity of a subspecies designation for a dwarf white sucker (*C. c. utawana*) based primarily on size differences should be reexamined.

BEAMISH, R. J. 1973. Determination of age and growth of populations of the white sucker (*Castostomus commersoni*) exhibiting a wide range in size at maturity. J. Fish. Res. Board Can. 30: 607–616.

On devrait éviter d'employer la scalimétrie pour déterminer l'âge des catostomes noirs communs adultes. Les coupes de rayons de la nageoire pectorale peuvent servir à déterminer avec précision l'âge de la plupart des populations, une fois le premier annulus identifié. L'âge des individus immatures peut être estimé par l'une ou l'autre méthode. Afin de faciliter l'identification du premier annulus du rayon de la nageoire et la détermination de l'âge des catostomes immatures, nous recommandons l'échantillonnage et de la nageoire pectorale et des écailles dans toute étude d'âge et de croissance de cette espèce.

Les catostomes noirs communs (*Catostomus commersoni*) examinés au cours de la présente étude atteignaient un âge de 17 ans, bien qu'il n'y ait eu que peu ou pas de croissance durant les dernières années. Nous avons observé des variations considérables de longueur à la maturité sexuelle dans toute l'aire de répartition de l'espèce. Pour cette raison, on devrait vérifier la validité du rang sous-spécifique, basé essentiellement sur des différences de taille, d'une forme naine (*C. c. utawana*) du catostome noir commun.

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IN a previous study (Beamish and Harvey 1969) it was shown that marks on sections of pectoral fin rays of the white sucker (*Catostomus commersoni*) formed annually and that ages obtained by the scale method could underestimate the true age of older individuals by as much as 5 years. Other authors (Dence 1948; Ovchynnyk 1965; Geen et al. 1966; Coble 1967) also have doubted the validity of ages determined from scales for the white sucker. This report elaborates on procedures for the determination of age of this species and discusses problems associated with the interpretation of fin-ray annuli from populations of white suckers exhibiting wide ranges in size at maturity. Growth curves from these populations determined by the pectoral fin-ray method have been included as earlier accounts of the growth of this species (Stewart 1926; Spoor 1938; Raney and Webster 1942; and others) were based on age determined by the scale method and should now be viewed with distrust.

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Materials and Methods

Populations of white suckers were extensively sampled from George and Lumsden lakes while less detailed investigations were made on populations from other

lakes. Growth data from populations from seven of these other lakes have been included in this report (Table 1). Most fish were captured in gillnets of 19–115-mm stretched mesh. In some populations small mesh trapnets (Beamish 1973) were used to obtain samples of smaller individuals. Sex, maturity, and fork length were recorded and a scale sample and at least the first three pectoral fin rays were taken from all fish. Scales were taken from the first and second rows above the lateral line just posterior to the base of the dorsal fin. The rays of the pectoral fin were cut as close to the body as possible using surgical bone cutters. The clean dry pectoral fin was covered with clear epoxy and hardened. A jeweler's saw was used to cut transverse sections close to the base of the rays. Larger fins were sectioned using a 6/0 or 7/0 blade and smaller fins with a 8/0 blade. It was usually convenient to cut three sections of about 0.5-mm thickness. In some instances it was necessary to cut sections of different thickness and at various distances from the base of the fin to determine the number of annuli. Individual sections were brushed clean and cemented to a microscope slide with "Permount" and xylene mixed in equal amounts. Under a compound microscope the fin-ray annulus appeared as an optically less dense zone in transmitted light.

Acetate impressions of scales or the original scale were viewed with a scale projector. The criteria described by Spoor (1938) were used to recognize the scale annulus, i.e., a crowding of circuli and cutting or crossing over of these crowded circuli at or near the point where they enter the posterior quadrant of the scale. In the anterior quadrant the annulus may be associated with irregularities or breaks in the circuli.

In George Lake 800 white suckers were tagged with numbered vinyl tubing (White and Beamish 1972). Comparisons were made between growth and circulus formation of young fish in the lake and white suckers hatched and reared in a laboratory. Laboratory white suckers were maintained in 16 ± 2 C dechlorinated tap water at the University of Toronto with a photoperiod of 13 hr. Fish were offered an ad libitum diet of a liver mixture. In July 1969 scales were taken from 41 of the laboratory-reared George Lake fish, which were then tagged and put back into the lake. Twenty of these fish had been caught in George Lake in September 1967 as young-of-the-year and then reared for 2 years in the laboratory. The remaining 21 were hatched from eggs fertilized in 1968 using George Lake white suckers and reared in the laboratory for 1 year. Before their release into George Lake in 1969 the first 3–5 pectoral rays were taken from one pectoral fin of the two-year-olds and five of the yearlings.

TABLE 1. Name and location of lakes in Ontario sampled for white suckers.

Lake	Location
George Lake	46°02′N,81°24′W
Lumsden Lake	46°01′N,81°26′W
Boulter Lake	45°18′N,78°03′W
Wadsworth Lake	44°51′N,77°34′W
Lake Simcoe	44°25′N,79°20′W
South Bay	45°34′N,82°00′W
South Pine Lake	45°43′N,82°06′W
Bridgland Lake	46°26′N,83°29′W
Franklin Lake	46°27′N,83°28′W

Identification of the fin-ray annulus from rapid-growing populations of white suckers

Identification of the first annulus in fin sections from rapidly growing white suckers was complicated by the presence of a false annulus. The smallest size-class captured in May and June 1967–70 from the George Lake population had one annulus on their scales but showed two possible annuli in their fin sections. The range and average length of these individuals was similar to the same stock hatched and reared in the laboratory for 1 year (Fig. 1). Individuals captured in George Lake and assigned age 2 by the scale method were slightly larger than those hatched and reared in the laboratory for 2 years. Genetically it would be possible for 12-month-old white suckers in George Lake to attain the sizes observed for George Lake individuals

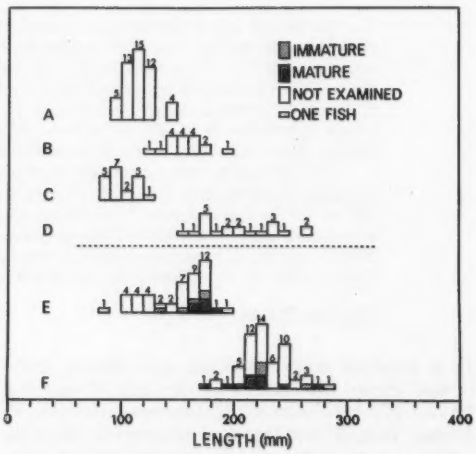


FIG. 1. Fork length frequencies of 1- and 2-year-old George Lake white suckers reared in the laboratory 1967–70, (A, B, C, D) with those captured in George Lake in 1968 (E,F). (A) measured April 1970, age 11 months; (B) measured June 1969, age 12 months; (C) measured April 1968, age 11 months; (D) same fish as in C, measured April 1969, age 23 months; (E) 1-year-old George Lake white suckers captured in the lake in May 1968; (F) 2-year-old George Lake white suckers captured in the lake in May 1968.

in the laboratory growth experiments. It was concluded that the scale age determination of 1 year was correct because fish that were assigned age 1 by scales were similar in size to laboratory-reared 1-year-old George Lake white suckers and because the fish in George Lake were the smallest that could be captured during the spawning period in May and June. The second mark in the fin sections was judged to represent the true annulus and the first mark was considered to be a false annulus.

The center of the finray in wild and reared young-of-the-year white suckers appeared as a clear crescent-shaped area (Fig. 2). In faster-growing individuals, dark material started to form around the center about 3 months after hatching. The first clear zone or false annulus developed 6–8 months after hatching (Fig. 3). An average of 10 circuli had been laid down on the scale by this time and some irregularity in circuli formation was evident (Fig. 3). This area of circuli irregularity was not an annulus in terms of the definition previously described and therefore was not labelled as a false annulus in Fig. 3. Patterns of circuli formation similar to this area of irregularity have been described as "accessory checks" by Hogman (1968) and others.

In older individuals the false annulus is not easily separated from the center of the ray (Fig. 4A, C). This is due, in part, to the difficulty in cutting sections close to the base of the rays. The first mark that is readily distinguishable outside the center of the ray in older fish from rapidly growing populations represents the first annulus and is not a false annulus.

The first scale annulus for George Lake white suckers formed in late June to early July after 20–40 circuli had formed (Fig. 4B, D). The true first fin-ray annulus was distinguishable by late May to early June (Fig. 4A, C). If allowance is made for the delayed appearance of the scale annulus, the ages determined by fins and scales are similar up to the start of spawning at age 4.

Three of the 41 white suckers of known age, introduced into George Lake in 1969, were recovered in May 1972. The fin-ray annuli corresponding to the years 1970 and 1971 were evident on all three and the new 1972 annulus was just visible on two. The yearly formation of the fin-ray annulus on these fish of known age adds confirmation to the observation that the fin-ray annulus forms as an annual event in George Lake white suckers (Beamish and Harvey 1969; R. J. Beamish unpublished data).

In a previous report (Beamish and Harvey 1969) the false annulus in fin sections and the corresponding irregularities on the scale for George Lake white suckers were incorrectly interpreted as representing the first annulus. Thus ages from both scales and fins were higher than the true age by 1 year. Fig. 3 from the 1969 paper has been corrected and included in this report as Fig. 5. The general conclusions of the 1969 report remain that ages obtained using scales and fin-ray sections are the same up to the time of maturity (at about age 4). After this time ages obtained using scale annuli are unreliable and may be in error by as much as 5 years for older individuals.

Identification of the fin-ray annulus from slow-growing populations of white suckers

A false annulus was difficult to distinguish in the fin sections of some slow-growing white suckers. Slow-growing Lumsden Lake white suckers developed an average of 14 circuli on the scale during their first year. These scale aged 1-year-old fish were the smallest size-class that could be captured shortly after the spawning period and were concluded to be 1-year-olds. The first mark clearly discernible in the fin-ray sections corresponded to the first scale annulus and was therefore considered to be be a true annulus. In other slow-growing populations a false annulus could be distinguished. In the Lumsden population it appeared that a clear zone or false annulus did from but that growth in the early months was insufficient to cause dark material to be deposited between the ray center and the false annulus.

In slow-growing white suckers the first true annulus forms at a distance from the ray centre that is similar to the position of the annulus in fast-growing fish. In older, slow-growing individuals the first annulus may become difficult to distinguish from the center of the ray. As was the case with the false annulus in the fast-growing individuals, this may be due, in part, to the difficulty in obtaining sections sufficiently close to the base of the ray. Thus, in fin sections from older slow-growing or "stunted" fish, the first easily distinguished mark may represent the second annulus. The first annulus in this case is closely associated with the ray center.

False annulus formation

A false annulus was apparent in the fin sections of laboratory-reared white suckers and some irregularities and crowding occurred in the circuli (Fig. 6A, B). Most fish reared from eggs in 1967–69 that were examined showed a false annulus although

not all had as distinct a mark as apparent in Fig. 6 A and B. Formation of the false annulus under laboratory conditions coincided with the estimated time of formation in lake dwelling young-of-the-year. Moreover, formation of a false annulus in fin sections and crowding of circuli on the scales (Fig. 6A, B) occurred without a change in photoperiod, temperature, or the ad libitum food ration. An annulus corresponding to a 1 year mark was not distinguishable in fin sections of laboratory-reared fish.

A synchronous decrease in growth rate occurred during January at an age of 8 months in white suckers hatched from eggs from four different lakes and reared in the laboratory in 1969–70.

It was not possible to test the relation of the formation of the false annulus and the synchronous decrease in growth rate because the experiments were designed to test another hypothesis. Hatchery-reared sockeye salmon (*Oncorhynchus nerka*) also showed a synchronous decrease in growth rate in all stocks during late summer despite the difference in acclimation temperatures of 5–20 C (Brett et al. 1969). No further change was noted up to the time of terminating the experiment 21 weeks later. Brett suggested that changes in the production of a growth hormone could account for such temperature-independent decrease in growth. Swift (1955) found that hatchery-reared underyearling brown trout (*Salmo trutta*) had a reduced growth rate in May and June despite increasing temperature and daylight.

The formation of the false annulus, the circulus irregularities, and the synchronous decrease in growth of the laboratory-reared white suckers appeared to be independent of temperature, photoperiod, and food ration. It is possible that the formation of the false annulus in underyearling white suckers from George Lake was also independent of these factors. This does not imply that all year marks are independent of the three factors mentioned, only that the mark designated as a false annulus in sections of fin rays and the corresponding check on the scales may in this instance result from some form of inherent physiological change.

Use of scales to determine age of older white suckers

Scales from some slow-growing white suckers may not show any distinct annuli (Dence 1948). Other studies have also shown that in older white suckers scale annuli become difficult to identify (Coble 1967; Geen et al. 1966). In older fish the number of circuli formed annually is reduced and crowding and cutting over of circulu at or near the point where they enter the posterior quadrant of the scale are no longer apparent. In many cases the only identifiable marks are irregularities in circuli formation in the anterior quadrant. By comparing the number of these irregularities found between identifiable adjacent annuli, it is possible to show that there is not an annual pattern in the formation of these irregularities and, hence, they should not be used to determine the age of the animal.

In general, the use of scales to determine the age of older white suckers can result in several types of errors: (1) if crowding and cutting over of circuli are used to identify an annulus, then an overestimate of the amount of yearly growth for older fish and an underestimate of the maximum ages in the population will usually be obtained; (2) if an annulus is identified by irregularities in circuli formation after crowding and cutting over of circuli are no longer apparent, an occasional accurate reading may be obtained for older suckers; however, there is little precision associated with this method of annulus determination; and (3) an error may result if a significant number of scales are rejected as not being readable. In this case the scales accepted usually come from faster-growing individuals, many of which (Table 2) are females (R. J. Beamish unplublished data). Growth differences within the population become obscured and, while an occasional accurate age may be obtained for the oldest individuals, the amount and rate of growth of the population may be overestimated.

Sections of pectoral fins have produced reliable estimates of age for lake-dwelling white suckers. However, neither pectoral fin sections nor scales have been acceptable in determining age of suckers inhabiting small streams and rivers. Suckers examined from these habitats usually lay down many clear zones and differentation of an annual mark is difficult (R. J. Beamish unpublished data).

Recommended method for determining the age of white suckers

In most instances scales provide the simplest method of age determination of immature white suckers but scales should not be used after the age of maturity. Pectoral fins and scales should be collected and processed from each specimen according to the procedures previously outlined. Fins may be allowed to dry in scale envelopes. If possible, samples should be obtained from the young-of-the-year. It is also helpful to know if the population is composed of fast-growing or slow-growing individuals. Because most white

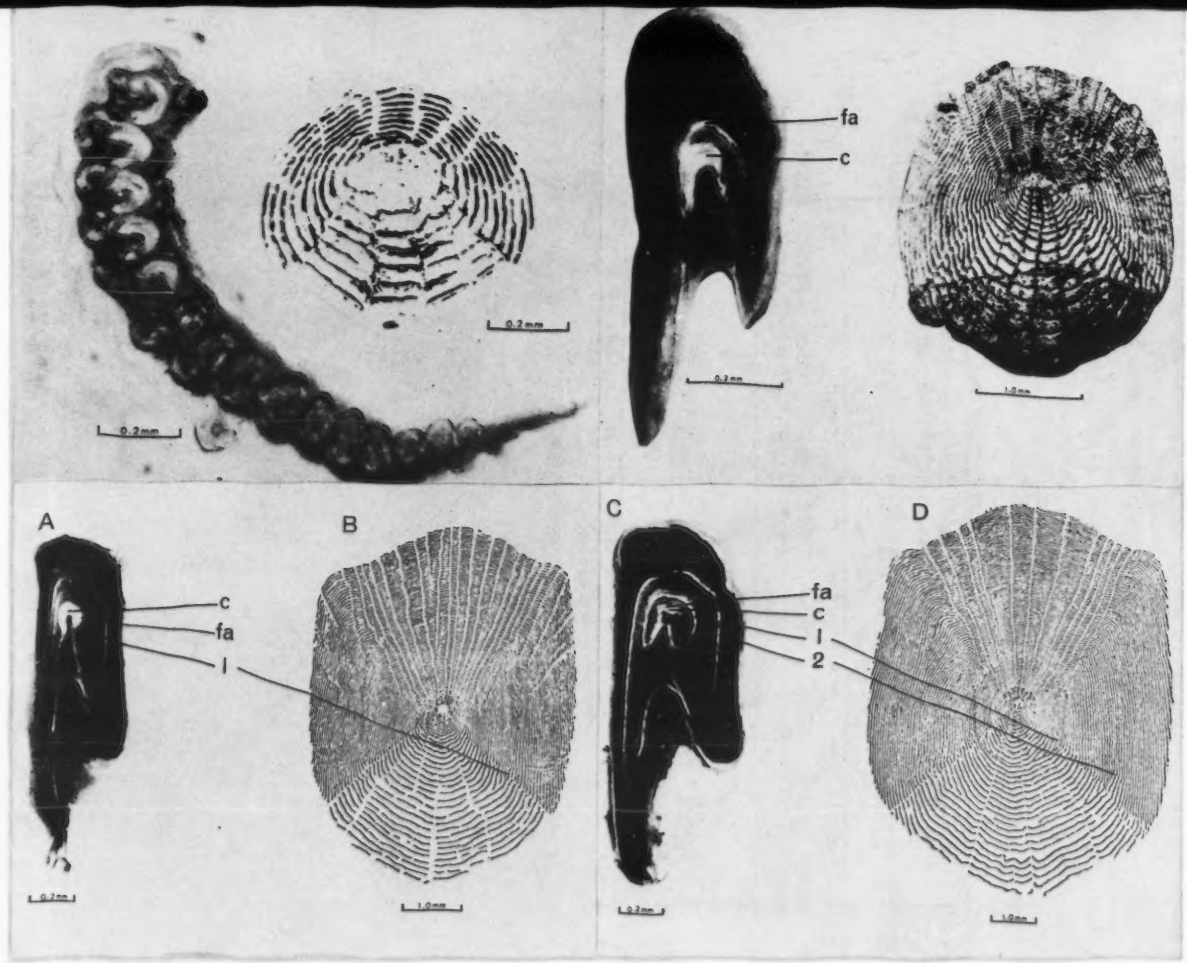
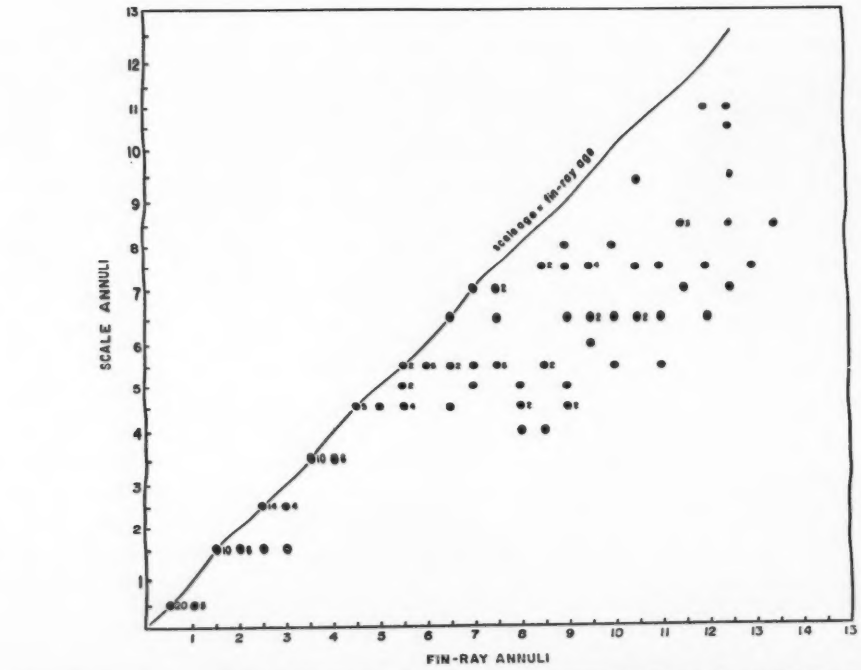


FIG. 2. (*top left*) Scale and transverse section of pectoral fin of a young-of-the-year white sucker, age 5 months.

FIG. 3. (*top right*) George Lake white sucker captured May 1968, age +, fork length 176 mm, weight 54 g. Fin-ray section prior to formation of first annulus (c) centre of ray, (fa) false annulus. Scale, showing circulus irregularities in the anterior quadrant after about 10 circuli had formed.

FIG. 4. (*bottom* A, B) Fin-ray section and scale from a George Lake white sucker age 1+, fork length 253 mm, weight 182 g captured October 1968. (C, D) Fin-ray section and scale from a George Lake white sucker, age 2+, fork length 318 mm, weight 440 g, captured May 1969. Scales photographed from acetate impression.



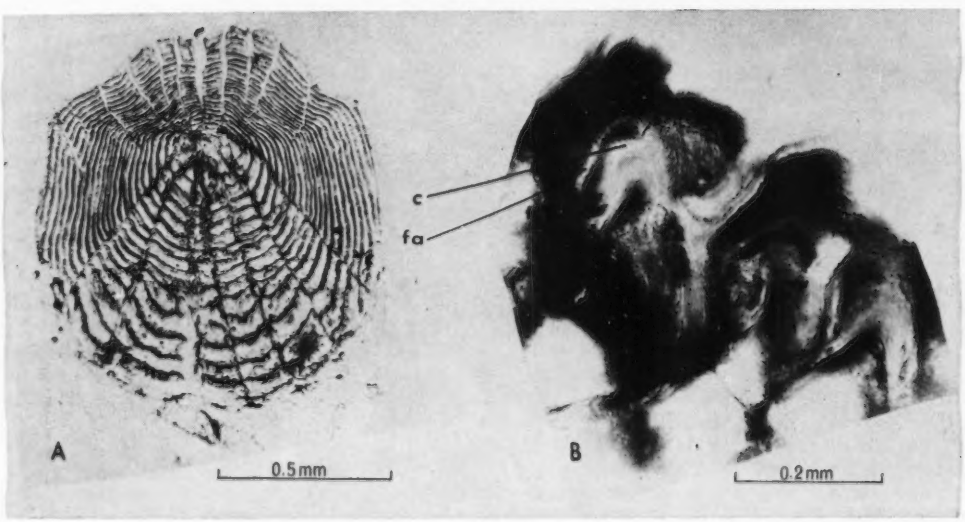


FIG. 5. (*top*) Relation between ages based on scale annuli and ages obtained from fin-ray annuli. Points are individual fish except where indicated.

FIG. 6. (*bottom*) Fin section and scale from laboratory-hatched and reared George Lake sucker, age 8 months.

sucker growth curves tend to flatten after the age of maturity, it can be estimated if a population is fast- or slow-growing by obtaining mature individuals and comparing their sizes with the growth curves presented in Fig. 7 and 8. The George and Lumsden lakes white sucker curves approximate the reported extremes in growth.

Before reliable estimates of age can be assigned, it is necessary to determine the position of the first annulus in the fin sections. This is most accurately accomplished by securing yearling white suckers. If reproduction has been successful each year, yearling white suckers will be found from the smallest size-class obtained during the spawning period. When such a sample cannot be obtained or there is some doubt concerning the size of yearling fish, then in most cases the position of the first fin-ray annulus may be estimated by comparison with the first scale annulus. Some irregularities and crowding of circuli occur on the scale at the time of false annulus formation in the fin rays. These irregularities occur after about 5–15 circuli

TABLE 2. Growth in length (millimeters) and weight (grams) of George Lake white suckers tagged in May 1967 and recovered from 1968–72. Fish tagged and released after being captured in gillnets have not been included in the table. (*No measurement available).

Spring									
1967		1968		1969		1970		1972	
length (mm)	weight (g)	length (mm)	weight (g)	length (mm)	weight (g)	length (mm)	weight (g)	length (mm)	weight[a] (g)
				Females					
438	1300	462	1860	474	1825	480	1800	*	*
461	1340	489	1670	503	1850	*	*	515	1975
468	1375	485	1650	504	1875	507	1900	*	*
472	1650	*	*	498	1975	507	1925	501	1675
475	1700	485	1965	485	*	497	2000	*	*
492	*	498	*	*	*	506	2100	*	*
506	1920	513	2400	*	*	516	2250	*	*
519	2020	528	2460	540	2425	541	2300	*	*
536	1940	543	2020	535	2140	537	2100	*	*
543	1965	553	2440	555	2225	*	*	563	*
552	2260	*	*	*	*	558	2550	*	*
569	2200	571	2585	*	*	*	*	573	2175
				Males					
tagged spring 1968		298	318	387	700	410	880	*	*
tagged Oct. 1968		300	367	316	430	378	750	*	*
445	1400	449	1640	455	1500	*	*	457	1360
452	1350	465	1600	471	1450	474	1480	*	*
456	1200	459	1300	462	1450	462	1450	*	*
457	1440	474	1726	473	1600	482	1600	*	*
464	1340	476	1550	*	*	484	1465	*	*
471	1425	475	1725	472	1500	475	1450	*	*
472	1600	*	*	482	1800	*	*	483	1340
481	1465	482	1470	484	1525	486	1560	*	*
482	1660	482	1485	487	1675	487	1575	487	*
489	1665	495	1365	495	1575	495	1550	*	*
491	1600	*	*	504	1625	*	*	504	1375
492	1620	489	2025	495	1650	491	1600	*	*
495	1600	*	*	500	1700	500	1725	500	1600
498	1650	496	1900	498	1775	498	1675	*	*
501	1800	504	*	504	1875	506	1875	*	*
511	1700	510	1720	513	1800	*	*	513	1430

[a]Decrease in weight in 1972 may be due in part to increasing acidic conditions within the lake (R. J. Beamish unpublished data; Beamish and Harvey 1972).

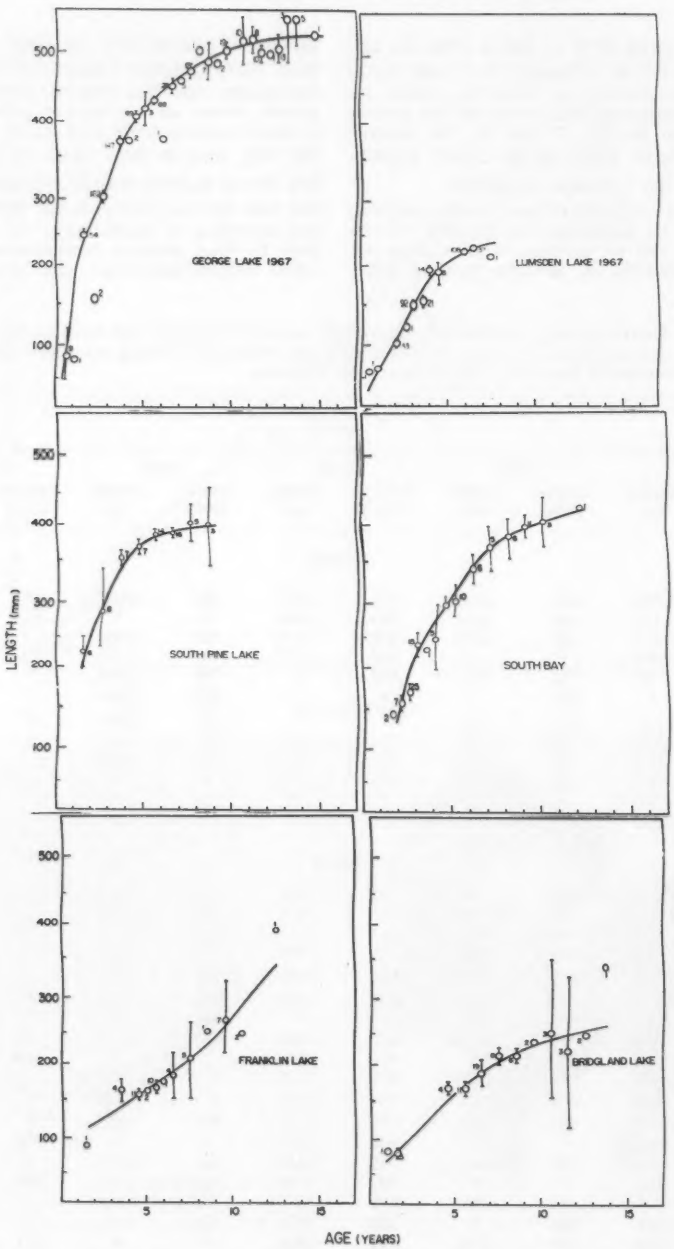


FIG. 7. Growth curves of age vs. mean fork length. Vertical bars represent the 95% confidence limits for each age-class. Numbers opposite each point indicate number of fish sampled in each age-class.

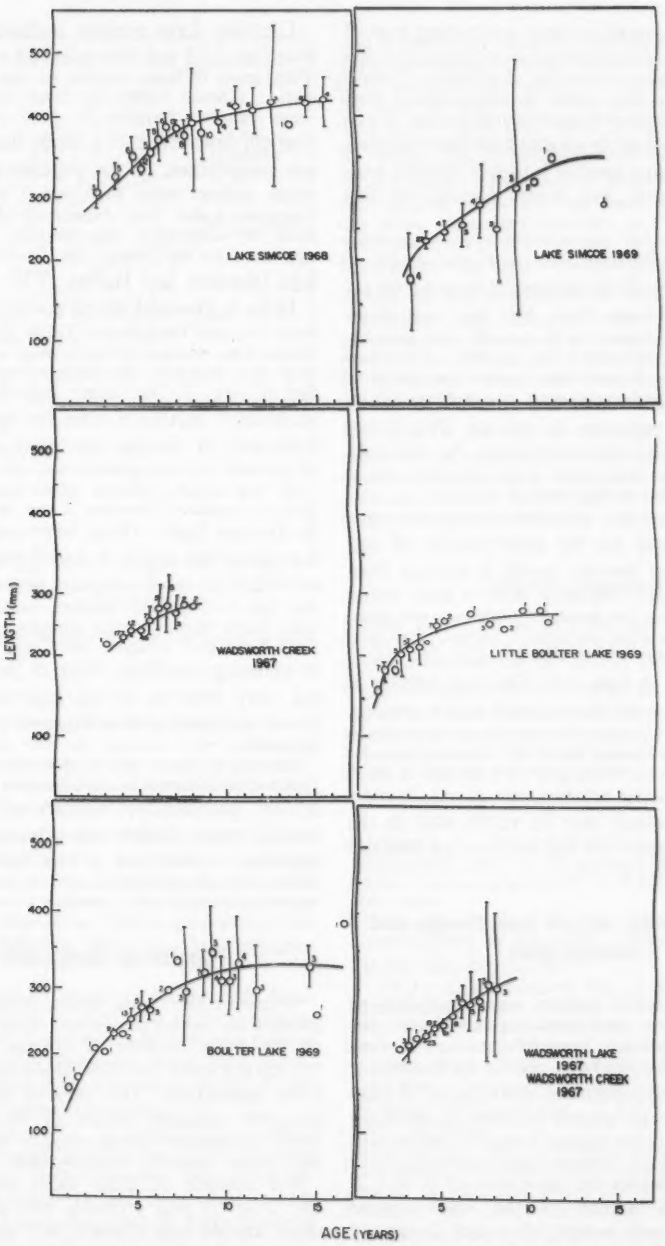


FIG. 8. Growth curves of age vs mean fork length. Vertical bars represent the 95% confidence limits for each age class. Numbers opposite each point indicate number of fish sampled in each age-class.

have formed but rarely is there any cutting over of circuli. In most of the populations examined to date the first scale annulus could be identified by distinct cutting over and thus could be differentiated from the area of circuli crowding and irregularities. If only older fast-growing white suckers have been sampled, then the first fin-ray annulus is usually the first well-defined clear zone that forms outside the first major dark zone.

The first annulus in older slow-growing white suckers is closely associated with the center of the ray and difficult to distinguish in some fin-ray sections. Scales from such fish may not show distinct annuli; hence, it is usually not possible to use scales to determine the position of the first fin-ray annulus. If yearlings cannot be captured from slow-growing populations and determinations to the nearest year are not critical, then it can be assumed that in older individuals the first well-defined clear zone that forms outside the first major dark zone represents the second annulus.

Only the first few pectoral fin-rays in older fish are acceptable for the determination of age, as the dark areas between annuli in sections from smaller rays are not sufficiently wide to allow differentiation of annuli. Occasionally both fast- and slow-growing white suckers develop a second clear zone ahead of and close to the true annulus; this occurs most frequently in ages 4–8. The true annulus is usually wider than this second mark and is continuous around the ray. Also, the spacing of dark material between this second mark and the true annulus is relatively uniform from year to year and is much less than the spacing between true annuli. In older individuals the annuli may be visible only in the faster growing area of the first few rays (see Beamish and Harvey 1969 Fig. 1B).

Growth of white suckers from George and Lumsden lakes

George Lake white suckers were rapid-growing fish attaining sizes approximating the largest described in the literature. Most fish matured in their fourth year (age 3+) and spawned for the first time at age 4. They reached maximum ages of up to 17 years although little or no growth occurred in the latter years, especially in the males (Table 2). The growth curve obtained from George Lake suckers in 1967 (Fig. 7) overestimates the mean size of 1- and 2-year-old suckers because of the small sample size. In 1968 a large sample of 1- and 2-year-old fish was obtained and the mean length of 148 mm for one-year-olds and 227 mm for two-year-olds was found.

Lumsden Lake suckers matured in their third year (age 2+) and first spawned at age 3 (Fig. 7). They grew to sizes similar to the so-called dwarf sucker (Dence 1948). In 1966 no 4-year-old fish were caught. Samples in 1967 contained no 5-year-old fish; hence, it is likely that the gap in the age composition of the population was real. No white suckers older than age 7 were captured in Lumsden Lake. The absence of older fish and the lack of successful reproduction in some years appeared to be related to conditions within the lake (Beamish and Harvey 1972).

Males in George Lake do not reach the maximum sizes attained by females (Table 2). Also, the maximum sizes reached by both sexes were not constant and this explains the variation in size for older fish in Fig. 7. The larger individuals in Table 2 maintained maximum sizes for several years and continued to develop sex products. This period of reduced- or non-growth was shorter for females.

A few small, mature white suckers of similar size to mature Lumsden Lake fish were present in George Lake. These were not slow-growing, but young fish age 1, 2, and 3 years, and of sizes equivalent to other immature white suckers of similar age in the lake. Mature, small males usually had freely flowing milt. Small-sized females with fully-developed ovaries were not observed to be in spawning condition. None of these small mature fish were observed in the spawning streams and it was not determined whether or not they actually spawned.

Several of the 1-and 2-year-old males that were hatched and reared in the laboratory also developed mature gonads. No estimate of percentage of mature white suckers was obtained, nor was it possible to determine if any females also were mature, as sacrifice of all mature fish in the growth experiments was not possible.

Populations from other lakes

White suckers from South Pine Lake (Fig. 7) showed an initial period of rapid growth similar to the white suckers of George Lake (Fig. 7), but did not reach the older ages found in the George Lake populations. This may be the result of the extremely shallow nature of South Pine Lake, which undergoes severe oxygen depletion during the winter months (Harvey and Coombs 1971).

The absence of older white suckers in South Bay (Fig. 7) was probably real and not due to small sample size. Coble (1967) studied this population and, although he did not age the fish, he showed that a decrease in larger individuals was due to lamprey predation. South Bay white

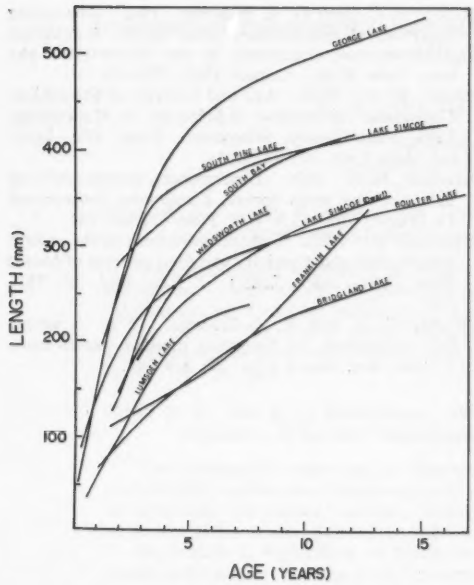


FIG. 9. Combination of growth curves of white suckers from 11 Ontario Lakes.

suckers are slow-growing; however, there was not a sharp decrease in rate of growth after maturity. Consequently, these fish attain larger sizes than other slow-growing forms such as in Franklin, Bridgland, and Lumsden lakes (Fig. 7)

Lake Simcoe has at least two distinct populations on the basis of fish size (Fig. 8). The faster-growing fish were captured at two sampling localities and had similar growth curves. The slower-growing population was obtained in 1969 in spawning condition from the Pefferlaw River, a short distance from the lake.

A similar situation exists in Wadsworth Lake and Wadsworth Creek (Fig. 8). During the spawning run into the outlet creek, some white suckers remain in the lake. Many of these are larger than creek-spawners of similar age and some of the larger individuals have immature gonads.

In Boulter Lake (Fig. 8) some white suckers migrated into a small shallow lake (Little Boulter Lake). A tagging study indicated that some individuals were resident in each lake while others moved from one lake to the other (B. Fallis personal communication).

A comparison of all growth curves (Fig. 9) indicates that white sucker populations in Ontario exhibit a wide range in size at maturity (age 3–5) and in maximum sizes and growth patterns. Because scale-age determinations were considered reliable up to the age of maturity, it was possible to compare the sizes of maturing white suckers in this study with those reported by other workers. Throughout its range, this species exhibited pronounced size differences at sexual maturity and, in some instances, considerable variation has been reported within the same watershed (Greeley and Bishop 1932). The range in growth in Fig. 9 probably occurs throughout the range of the species and is not unique to populations in Ontario.

Different size-classes of white suckers in the same lake were reported in this study and by Dence (1948). Dence recognized the slower-growing form as a distinct subspecies *Catostomus commersoni utawana* (Mather). The occurrence of extensive variation in the size at maturity throughout the range of the white sucker casts considerable doubt on the validity of a size-based subspecies designation. A study of the validity of this subspecies designation is currently in progress.

Acknowledgments

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Evidence of Interspecific Interactions in the Respiration of Tubificid Oligochaetes

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CHUA, K. E., AND R. O. BRINKHURST. 1973. Evidence of interspecific interactions in the respiration of tubificid oligochaetes. J. Fish. Res. Board Can. 617–622.

Determination of respiration of *Tubifex tubifex*, *Limnodrilus hoffmeisteri*, and *Peloscolex multisetosus* in the laboratory provided evidence of interspecific interactions between two tubificid species. The same individuals in mixed species culture respire significantly less than in pure culture. The effector of the interaction in the one interaction between *T. tubifex* and *L. hoffmeisteri* is waterborne, smaller than 0.45 μ, and it is rendered ineffective by heating at 60 C for 20 min.

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Des mesures de respiration au laboratoire sur *Tubifex tubifex*, *Limnodrilus hoffmeisteri*, et *Peloscolex multisetosus* démontrent qu'il y a interaction interspécifique entre deux espèces de tubificidés. Les mêmes individus, placés en culture d'espèces mixtes, ont une respiration nettement moins élevée qu'en culture pure. L'agent responsable de l'interaction, dans le cas de *T. tubifex* et de *L. hoffmeisteri*, est transporté par l'eau, a une taille inférieure à 0.45 μ, et est rendu inefficace par chauffage à 60 C pendant 20 min.

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EXPERIMENTALLY determined respiration rates, together with estimates of ingestion, egestion, and growth in energy terms, have been used in production studies and in the derivation of various ratios such as growth efficiency. The validity of many attempts to use the balanced energy equation may be questioned because it is commonly solved for at least one unknown. The equation itself may be oversimplified by the omission of a number of energy losses such as secretions and fluid excreta. Respiration is one commonly measured variable, and the importance of methodology (particularly in respect to temperature, acclimation, substrate, illumination, vibration, activity, and other factors that can modify it) has often been investigated, though this knowledge has not always been applied by ecologists. A further unlooked for complication was discovered during a study of respiration, growth, and feeding of three tubificid oligochaete species (*Tubifex tubifex*, *Limnodrilus hoffmeisteri*, and *Peloscolex multisetosus*). When these three species were mixed, the growth and respiration rates differed from the sum of the rates obtained from the same species in pure culture. As a result, assimilation (i.e. the sum of respiration and growth) by these species in pure culture was about half of that obtained using mixed cultures (Brinkhurst et al. 1972).

In that earlier study, the effect of mixing pairs of species was investigated in relation to growth but not for respiration. The presence of *P. multisetosus* increased the weight gain of the other two species by a factor of 2–3, although it was seemingly unaffected. The respiration rate of a mixed culture of all three species was only two-thirds the rate calculated from the sum of the rates obtained using the same individual worms in pure culture both before and after mixing them.

We are now able to report on 1–1 respiratory interactions among these three species. We also employed a different and more sensitive method of measuring dissolved oxygen, and undertook some preliminary experiments to elucidate the mechanism by which worms of one species detect the presence of those of another species.

Materials and Methods

Specimens of *T. tubifex*, *L. hoffmeisteri*, and *P. multisetosus* were collected from Toronto harbor with an Ekman grab and were kept at 15 C for at least 3 months prior to use. The average dry weights per worm were

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0.30, 0.41, and 0.09 mg, respectively. Presterilized respiratory vessels (6 × 1.5-cm diameter) containing 2.5-ml washed sand (0.25-mm particle size) and 7.0-ml air-saturated water were kept in a water bath at 15 C. The sand contained an additional 0.5-ml water. Sterile vaccine stoppers were inserted without trapping air bubbles. A 1-ml tuberculin syringe was used to extract water samples through the stopper, and oxygen concentrations in the vessel were measured using an IL-*p*H Blood Gas Analyser model 113–51, standardized before each run with 12% oxygen as the primary gas, an oxygen-free gas, and air. The readings, in millimeters Hg, were converted to percent saturation and then to microliters of oxygen using a nomogram. The water bath and analyser sample chamber were connected to a Haake Constant Temperature Circulator maintained at 15 C throughout. Between 10 and 20 worms were placed in each respiratory vessel and after 90 min each vessel was removed from the water bath, inverted once, and 0.4 ml of water sample was withdrawn by pushing gently on the cap, thus displacing water into the syringe and avoiding the formation of air spaces. The sample was injected slowly (without introducing air) into the analyser sample chamber in three aliquots and the average was used as the initial oxygen value. The vessel was returned to the bath immediately after the water sample was taken and was held there for another 2½ hr before the second readings were made using the same procedure. The oxygen consumed by control vessels without worms was subtracted from the difference between initial and final readings to give respiration values for worms during the time elapsed.

Experiment A was set up using the species in pairs so as to determine each possible interaction between the three species used. Four vessels were used for each experimental run. On the first day, 10 worms of one species were placed in one vessel, 10 of a second species in another vessel, and 10 of each separated by a "Nitex" nylon monafilament screen (pore size 0.1 mm) in the third. The fourth vessel contained no worms and was used as a control. On the second day, the worms from the pure cultures were placed in the divided vessel, and vice versa. Each experiment therefore yielded two estimates of the respiration rates of the worms in pure and mixed culture, each of which involved the same individual worms. In one instance the respiration rate in mixed culture was determined prior to that in pure culture and, in the other, the reverse procedure applied. The whole operation was repeated three times for each pair of species. The flow chart for these experiments is illustrated in Figure 1A.

Between the two parts of each run, the worms were kept overnight in tubes containing sediment and dechlorinated tap water at 15 C, being recovered the next day using a 0.25-mm sieve. At the conclusion of the whole run, the worms were recovered as before and placed in different tubes in dechlorinated tap water and held overnight at 15 C to allow the gut contents to be voided. They were then dried on planchets at 70 C for 12 hr and weighed on a Cahn electro-balance. The dry weights recorded were then summed a) according to species to determine the rates of oxygen consumption in pure culture, and b) according to respiratory vessels occupied by mixtures of two species.

The fact that the mixed cultures contained twice as many worms as the pure cultures was not considered likely to affect the interpretation of results because no effect of density on respiration rate was found in an earlier test in which the population was increased by a factor of 3 (Brinkhurst et al. 1972).

The next set of experiments (B) was carried out using only *T. tubifex* and *L. hoffmeisteri*. Three vessels were used, one containing 10 *T. tubifex*, the second containing 10 *L. hoffmeisteri*, and the third had no worms. Respiration rates for these worms in pure culture were determined as before, but, on the second day, instead of mixing the two species, water from a culture of *T. tubifex* (kept in 30-ml sterilized dechlorinated tap water in a flask for 2 hr at 15 C) was substituted for the water in all three vessels (Fig. 1B).

In the final series (C), the number of vessels containing worms was doubled so that five vessels were used in each

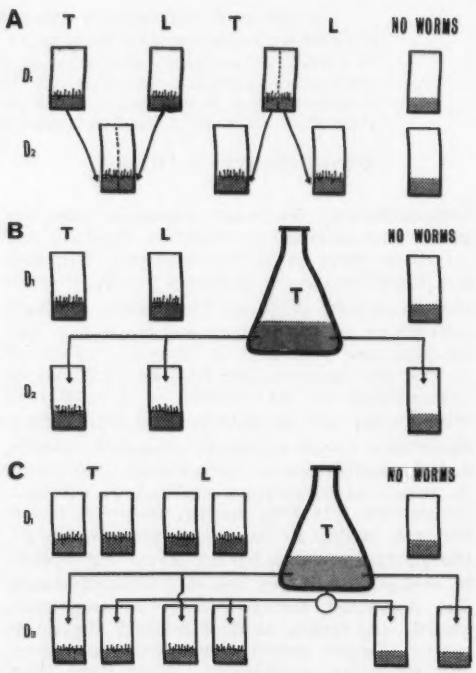

FIG. 1. Schematic representation of the experimental design. D_1 and D_2 = day 1 and day 2, respectively, T = *Tubifex tubifex*, L = *Limnodrilus hoffmeisteri*. In A, dotted lines represent a screen. In B and C, the flasks represent *T. tubifex* water. In C, the ○ represents treatments by either filter or heat. In experiment A the procedure was repeated using *Peloscolex multisetosus* with *T. tubifex*, and with *P. multisetosus* and *L. hoffmeisteri*.

run. In this series (Fig. 1C), half of the water from the culture of *T. tubifex* was either passed through a Millepore filter (0.45-μ pore diameter) or filtered and heated at 60 C for 20 min, these series being identified as C′ and C″, respectively. Untreated water was added to one set of vessels containing each species, treated water being added to the other. Hence, experiment B, repeated only three times, was eventually rerun another six times in C′ and six times in C″.

Results

When the respiration rates derived from all of the single species cultures in experiments A are combined into two 6-set series (Table 1), an analysis of variance demonstrated no significant differences between series within species so that we can regard the rate estimates as constant throughout. When the overall mean respiration rate for each species was calculated, significant differences were revealed between species ($P < 0.01$ in a t-test). The lowest respiration rate was that of *L. hoffmeisteri*, the highest that of *P. multisetosus*, although the difference between that of the latter and *T. tubifex* was not apparent in the second 6-set series. This discrepancy was not tested by analysis of variance because the species were actually used in pairs rather than in the three-way arrangement suggested by the layout of the table.

TABLE 1. Rates of oxygen consumption (microliters/mg dry weight/hr) at 15 C for three tubificid species in pure culture.

	Tubifex tubifex	*Limnodrilus hoffmeisteri*	*Peloscolex multisetosus*
	Series A		
	0.64	0.36	0.67
	0.45	0.55	0.70
	0.54	0.35	0.54
	0.56	0.36	0.41
	0.61	0.49	0.80
	0.52	0.49	0.61
$\bar{x}$	0.55	0.43	0.62
	Series B		
	0.65	0.58	0.77
	0.61	0.47	0.44
	0.66	0.23	0.64
	0.61	0.32	0.62
	0.61	0.39	0.60
	0.52	0.48	0.55
$\bar{x}$	0.61	0.41	0.60
Overall $\bar{x}$	0.58	0.42	0.61

Respiration rates of *T. tubifex* in pure culture in experiments B and C (Fig. 1) ranged 0.50–0.54 µliter/mg/hr, while those of *L. hoffmeisteri*, when not affected by the experimental procedure, ranged 0.37–0.43 µliter/mg/hr, both being somewhat lower than the average values obtained in pure culture in experiment A.

In every section of experiment A in which two species were separated only by the Nitex screen, the respiration rates of the pairs were significantly lower than the sums of the respiration rates of the same individuals in pure culture ($P < 0.01$ for a two-way analysis of variance on data in Table 2). The reductions in respiration rate observed were of the same magnitude in each instance (no significant differences using one-way analysis of variance).

The unfiltered, unheated water from the *T. tubifex* culture (experiment B) failed to affect the respiration rate of that species in pure culture ($P > 0.99$ on data from Table 3), but it significantly affected that of *L. hoffmeisteri* ($P < 0.001$ on data from Table 4). The degree of reduction observed was similar in each instance. Filtering the culture water from *T. tubifex* (experiment C′) failed to affect its ability to reduce respiration in *L. hoffmeisteri*, the average reduction being the same as before ($P < 0.0001$). When the culture water was heated (experiment C″), the effect was destroyed so that there was no significant change in respiration rate of *L. hoffmeisteri* from that observed in tap water ($P > 0.89$). Neither treatment made the culture water effective in altering the respiration rate in *T. tubifex* itself. No attempt was made to determine the effect of *Limnodrilus* culture water on *Tubifex* or any interaction involving *Peloscolex*.

Discussion

Our method of determining oxygen utilization, although lacking observation of the variation of rate of uptake during the experimental period, paid careful attention to conditions preferred by tubificid oligochaetes, i.e. they were kept in the dark, in a substrate, and free from excessive vibration or shaking. Our calculations were based on the volume of water in the vessels (7 ml) rather than the total including interstitial water (7.5 ml), the use of which would increase our final values by 7%. The interstitial water was mixed with the rest by inverting the chambers at T_1 and T_2 rather than by relying on the stirrers fitted to the BOD probes used in the earlier study (Brinkhurst et al. 1972) in which we reported somewhat lower mean respiratory values (0.4, 0.3, and 0.4 as opposed to 0.51–0.61, 0.37–0.43, and 0.60–0.62 µliter/

mg/hr at 15 C for *T. tubifex*, *L. hoffmeisteri*, and *P. multisetosus*, respectively). Johnson and Brinkhurst (1971) used BOD probes with oscillating agitators, rather than the later-model rotary stirrers, and obtained values of 1.04 and 0.59 µliter/mg/hr for *T. tubifex* and *L. hoffmeisteri*, values significantly higher than those obtained here, as were the values obtained at 5, 10, and 20 C compared to those obtained at these temperatures by Brinkhurst et al. (1972).

Most earlier authors obtained higher values than we did for these two cosmopolitan, common tubificids. The relation between respiratory rate and temperature is not consistent with constant Q_{10} changes (Brinkhurst et al. 1972) so that we cannot compare published results in relation to a constant temperature, but we have converted them to the same units as those used herein. Berg et al. (1962) obtained a value of 0.4 for *T. tubifex* at 11 C and a value of 1.15 at 20 C can be calculated from data presented by Ivlev (1939), both of which are in reasonable agreement with our results, but those of Fowler and Goodnight (1965) and Palmer (1968) using Warburg methods are exceedingly high (3.5 at 25 C, 3.4 at 20 C, respectively). Calculations from data by Teal (1957) on *L. hoffmeisteri* yielded a value of 1.67 µliter/mg/hr at 10 C, or about eight times the value obtained by Brinkhurst et al. (1972), although lack of detail and internal inconsistencies in data make our calculation of this value questionable. Dausend (1931) obtained a value of 0.6 at 19 C using unidentified tubificids, perhaps a mixture of species. Few other tubificid species have been investigated, despite the need to know the difference between tolerant and intolerant species commonly used as pollution indicators. The warmwater species *Branchiura sowerbyi* used 0.7 µliter/mg/hr at 20 C in a tube (in lieu of a burrow), 0.88 without a tube (Aston 1966). *Psammoryctides* (as *Tubifex*) *barbatus* used 0.3 at 11 C and *Potamothrix* (as *Ilyodrilus*) *hammoniensis* consumed about the same amount at 12 C according to Berg et al. (1962), both values being roughly equivalent to those determined by us.

While there are bound to be variations in laboratory-determined respiration rates dependent upon

TABLE 2. Rates of oxygen consumption (microliters/mg dry weight/hr) for three tubificid species in pure culture and as pairs separated by a screen (Experiment A). T = *T. tubifex*, L = *L. hoffmeisteri*, P = *P. multisetosus*.

Species	Sum of rates determined separately	Rates in combined culture	Reduction in respiration rate observed in combined culture (%)
L and P	0.47	0.24	49
	0.61	0.39	36
	0.40	0.24	40
	0.37	0.26	30
	0.57	0.47	18
	0.52	0.38	27
$\bar{x}$	0.49	0.33	33
T and P	0.66	0.33	50
	0.45	0.31	31
	0.56	0.32	43
	0.58	0.30	48
	0.61	0.35	43
	0.52	0.33	37
$\bar{x}$	0.56	0.32	43
L and T	0.61	0.38	38
	0.54	0.20	63
	0.37	0.24	35
	0.43	0.21	51
	0.49	0.26	47
	0.49	0.25	49
$\bar{x}$	0.49	0.26	47

acclimation and methodology used, the percent reduction in respiration observed in mixed cultures (33, 43, and 47 for species pairs, 34 and 29 for *L. hoffmeisteri* in unfiltered and filtered *Tubifex* culture water, and 31% for mixtures of all three species used earlier) is large, the rates being significantly different from the mean values for the same species held in pure culture or in tap water. There are no significant differences in the degree of reduction noted in any of the experiments, which suggests

TABLE 3. Rate of oxygen consumption of *T. tubifex* (microliters/mg/hr) at 15 C in tap water and water from a culture of *T. tubifex*. (Percent equals culture/tap × 100, i.e. 100% equals no change.)

Experiment (*Fig. 1*)	Untreated water			Filtered water			Heated water		
	Tap	Culture	%	Tap	Culture	%	Tap	Culture	%
B 1	0.67	0.62	93						
2	0.56	0.55	98						
3	0.51	0.48	94						
C′ 1	0.55	0.58	105	0.45	0.45	100			
2	0.55	0.50	91	0.57	0.57	100			
3	0.55	0.55	100	0.55	0.54	98			
4	0.59	0.59	100	0.59	0.54	92			
5	0.63	0.67	106	0.58	0.53	91			
6	0.59	0.54	92	0.42	0.42	100			
C″ 1	0.49	0.53	108				0.47	0.46	98
2	0.41	0.39	95				0.55	0.57	104
3	0.56	0.59	105				0.42	0.44	105
4	0.47	0.51	108				0.52	0.53	102
5	0.49	0.49	100				0.55	0.53	96
6	0.54	0.57	106				0.51	0.51	100
$\bar{x}$	0.54	0.54	101	0.53	0.51	97	0.50	0.51	101

TABLE 4. Rate of oxygen consumption of *L. hoffmeisteri* (microliters/mg/hr) at 15 C in tap water and water from a culture of *T. tubifex*. (Percent equals culture/tap × 100, i.e. 100% equals no change.)

Experiment (*Fig. 1*)	Untreated water			Filtered water			Heated water		
	Tap	Culture	%	Tap	Culture	%	Tap	Culture	%
B 1	0.27	0.16	59						
2	0.42	0.26	62						
3	0.41	0.25	61						
C′ 1	0.34	0.19	56	0.42	0.29	69			
2	0.37	0.24	65	0.44	0.33	75			
3	0.35	0.25	71	0.41	0.25	61			
4	0.27	0.20	74	0.43	0.32	74			
5	0.42	0.30	71	0.48	0.39	81			
6	0.41	0.32	78	0.41	0.28	68			
C″ 1	0.41	0.28	68				0.39	0.42	108
2	0.38	0.30	79				0.35	0.36	103
3	0.39	0.24	62				0.55	0.55	100
4	0.34	0.23	68				0.36	0.36	100
5	0.35	0.22	63				0.30	0.32	107
6	0.35	0.18	51				0.33	0.31	94
$\bar{x}$	0.37	0.24	66	0.43	0.31	71	0.38	0.39	102

that all the species are reacting in the same way. Hence, the application of laboratory values for respiration to field situations in relation to pollution tolerance or studies involving solution of the balanced energy equation for estimation of benthic production should recognize the possibility of interspecific interactions.

The interactions in growth and respiration detected by these experiments can be understood in relation to the biology of sludge worms. We know that 72% of at least one group of the bacteria passing through the gut of a tubificid are destroyed, and that tubificid species differ in their ability to digest a number of heterotrophic aerobic bacteria (Wavre and Brinkhurst 1971). The fresh feces of each worm species differ in their bacterial population, and it is possible that recolonization initially favors the build-up of populations of those bacterial species making up the bulk of the surviving 28%. The worms also ingest sedimentary material selectively, as indicated by experiments in which feces contain more organic material, nitrogen, and calories than the sediment presented as food (Brinkhurst et al. 1972).

All of this suggests that the feces of one worm species might be the preferred food of a second species from the same habitat. Selective ingestion and specialization in digestive abilities provide two possible bases for niche discrimination in organisms lacking marked physical adaptations but which form mixed colonies of as many as twelve species.

Tubificid colonies are tightly clumped, both in the field and in laboratory cultures, but these clumps are themselves transient, at least in aquaria. It is possible to see how worms could be adapted to seeking out worms of another species through the recognition of dissolved metabolites released with the excreta. Worms in pure culture might therefore be expected to be more active than those in mixed culture so that respiration would be depressed and growth increased in the mixed culture. The gain in growth in mixed culture exceeds that which can be attributed to a lower cost of respiration, which can be explained if feeding replaces searching activity, particularly if feeding by each species in a mixed culture is concentrated on preferred food, i.e. that sediment containing a high proportion of the bacteria most easily digestable. Defecation rate of mixed populations is, in fact, 24% higher than that observed in pure culture (Brinkhurst 1972).

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Evaluation of a Method for Estimating Food Consumption Rates of Fish[1,2]

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CARLINE, R. F., AND J. D. HALL. 1973. Evaluation of a method for estimating food consumption rates of fish. *J. Fish. Res. Board Can.* 30: 623–629.

A method of estimating food consumption rates of fish in nature from laboratory growth data was evaluated using juvenile coho salmon (*Oncorhynchus kisutch*) in three similar experiments. One group of coho was held individually in aquariums where movement was restricted. Another group was maintained in an experimental stream where the coho displayed many of their typical behavior patterns. All fish were fed known rations and both groups had similar growth efficiencies over a wide range of rations. Coho feeding at intermediate rates had the highest gross efficiencies. Aggressive activity did not affect growth efficiency. Results suggested that laboratory food and growth data may provide reasonably accurate estimates of food consumption of coho salmon in nature.

CARLINE, R. F., and J. D. HALL. 1973. Evaluation of a method for estimating food consumption rates of fish. *J. Fish. Res. Board Can.* 30: 623–629.

Les auteurs évaluent une méthode d'estimation des taux de consommation alimentaire par des poissons dans la nature, à partir de données recueillies au laboratoire. Ils utilisent à cette fin des jeunes saumons coho (*Oncorhynchus kisutch*) dans trois essais semblables. Un groupe de saumons coho ont été maintenus individuellement dans des aquariums, où leurs mouvements étaient restreints. Un autre groupe a été gardé dans un cours d'eau expérimental, où les saumons ont démontré, en général, un comportement typique. Tous les poissons ont été nourris d'un régime connu, et les deux groupes ont démontré la même efficacité de croissance sur une gamme étendue de rations. Les saumons coho à taux d'alimentation intermédiaire ont la plus haute efficacité brute. Un comportement agressif n'affecte pas l'efficacité de croissance dans le cours d'eau expérimental. Ces résultats suggèrent que les données recueillies au laboratoire sur l'alimentation et la croissance peuvent fournir une estimation assez juste de la consommation d'aliments par le saumon coho dans la nature.

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ONE of the key elements in growth models of fish populations is food consumption, a difficult parameter to measure in nature. Davis and Warren (1968) have suggested that the food consumption of fish in nature may be best estimated by extrapolating from laboratory-derived curves of food consumption and growth rate. To use this method one must assume that fish in the laboratory have the same energy requirements for activity as fish in nature, or at least that they have similar growth efficiencies. The problems associated with this use of laboratory data have been considered by others, including Allen (1951), Horton (1961), and Kerr (1971).

Our study, an attempt to bridge the gap between laboratory and field approaches, sought to evaluate this method of estimating food consumption rates of fish in nature. Groups of juvenile coho salmon (*Oncorhynchus kisutch*) were held in an outdoor stream tank and fed in such a way that their food consumption could be well defined. Their behavior included the full repertoire of aggressive activity noted in wild coho. The growth rates and food conversion efficiencies of these fish were compared to those of coho held individually in small aquariums that restricted activity. Coho salmon were chosen because their biology and social behavior were well known from studies in other experimental streams (Chapman 1962; Mason 1969).

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Materials and Methods

The experiments were conducted in a stream tank with glass walls and in adjoining aquariums. The stream tank was divided in half by a partition with openings at the ends, permitting a circular flow of water (Fig. 1). Two paddlewheels propelled the water in the channels. Each half of the tank consisted of two riffles and two pools, with a sloping surface connecting the riffles and pools. Bottoms of the channels were covered with washed gravel. Water velocity was 7 cm/sec on the riffles and decreased to less than 3 cm/sec in the pools.

Screens at the upstream and downstream end of each half of the tank confined the fish to an area of 4.5 m^2. Small screen cages were connected to the downstream screens to provide a refuge for any subordinate fish that might be driven out of the section. A 0.16-m^2 screen cage was kept in the vacant area adjacent to one of the paddlewheels to hold fish that were to be starved. Well water was exchanged through the system at 4 liters/min, or 8% of the tank volume per hour.

The only foods available to the fish were larvae of the common housefly (*Musca sp.*), introduced daily in known amounts. The larvae were washed down a series of glass tubes that extended from the sides of the tank to the water surface. The entire structure was covered by fiberglass window screening to exclude terrestrial organisms.

The aquariums, made of clear plexiglass, were 18 × 23 × 25 cm. Exchange water from the stream tank was supplied to each aquarium at 60 ml/min. The aquariums were also covered with screening.

Juvenile coho were seined from nearby coastal streams and held in outdoor tanks prior to each experiment. Each fish was given a distinctive mark by cold branding (Everest and Edmundson 1967). At the beginning of each experiment fish were starved for 48 hr, narcotized, measured (fork length) to the nearest millimeter, blotted dry, weighed to the nearest milligram, and placed in stream channels or aquariums.

Fly larvae were usually fed to the stream fish twice daily. The food consumption of each fish was determined by counting the number of larvae it consumed. Several samples of fly larvae were taken during each experiment to determine average dry weight. Water temperatures were taken twice daily with a stem thermometer accurate to 0.5 C.

During the first experiment, the behavior of the stream fish was observed for 10 min prior to and during each feeding. Behavioral observations were confined to the prefeeding period in the two following experiments. Each time an aggressive act was observed, the initiator of the act and the recipient were noted. In the final analysis, acts initiated and received were combined as an index of aggressive activity for each fish. Levels of aggressive activity were lower during feeding than they were prior to feeding; we have used only those observations made before feeding (Table 1). No distinction was made between threat displays, chases, or nips (Chapman

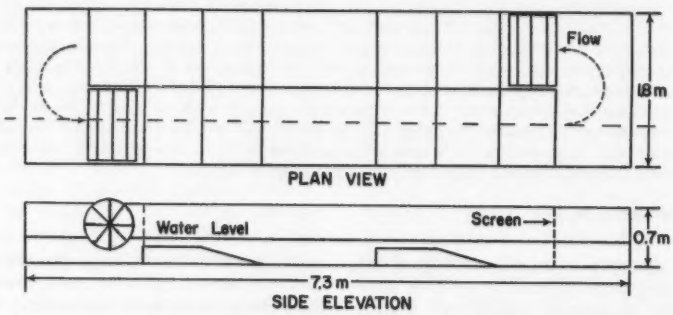

FIG. 1. Top view and cross-sectional side view of observation tank. Windows on the sides of the tank are not shown.

TABLE 1. Experimental conditions, fish sizes, and summary of aggressive activity. Data are mean values with ranges in parentheses.

Experiment	Date 1967	Water temperature (C)	Fish weight (g)	Fork length (mm)	Aggression acts/fish/min
I	19 Feb.–4 Mar.	5.9 (3.3–8.2)	6.32 (4.10–7.38)	78 (71–81)	0.27 (0–1.11)
II	24 Mar.–6 Apr.	7.2 (5.0–9.0)	0.86 (0.66–1.25)	45 (41–51)	0.54 (0–2.85)
III	16 May–2 June	12.3 (10.0–14.5)	0.91 (0.59–1.42)	46 (40–53)	0.49 (0–1.44)

1962). If a threat display was followed by a nip, it was counted as a single aggressive act. The position of each fish in the stream was recorded at each observation.

Aquarium experiments were conducted concurrently with stream experiments. Eleven of the aquarium fish were fed predetermined numbers of fly larvae from below the maintenance ration up to the maximum consumption rate; three fish were starved. Uneaten larvae were removed from the aquariums each day just prior to feeding. Additional information about each experiment is summarized in Table 1.

At the end of each experiment, all fish were starved for 48 hr, weighed and measured, dried in an oven at 65 C for 5 days, and reweighed. Any fish showing external signs of disease were not included in the results.

Food consumption and growth rates for each fish were computed by dividing the dry weight of food consumed or the change in dry weight of the fish by the mean dry weight of the fish. The quotient was then divided by the number of days in the experiment. To estimate the initial dry weight of each fed fish, its initial wet weight was multiplied by its final percentage dry weight. We felt that the variability in the relation between feeding level or condition factor and percentage dry weight did not justify a correction for feeding level or change in condition factor. To estimate initial dry weight of each starved fish, its initial wet weight was multiplied by the mean percentage dry weight of the fed fish. The final percentage dry weight of starved fish was not used to estimate initial dry weights because percentage dry weight of starved fish decreases with time (Brett et al. 1969).

Curves depicting the relation between food consumption and growth rate were fitted by the method of least-squares regression analysis. A linear relation was assumed unless a statistically significant reduction in the residual sum of squares resulted from fitting a curvilinear relation. Gross efficiencies were calculated by dividing change in weight by total weight of food consumed (Warren and Davis 1967). Net efficiencies were calculated by dividing change in weight by weight of food consumed less the maintenance ration.

Results

Experiment I.

Two days after the coho were placed in the stream channel they had established territories and hierarchies. The distribution of fish in both channels was similar. A dominant fish defended the entire upstream half of the channel and the other 10 fish remained in the downstream section. Any fish entering the upper section was quickly chased downstream.

A dominant fish in the lower half of the channel defended the area between the riffle and pool. Any subordinate fish that tried to come alongside or get in front of the dominant fish was chased back into the pool. A subordinate fish took up a position 15–30-cm downstream from the dominant fish and defended this area against the remaining subordinates. It appeared that a hierarchy developed among the rest of the subordinate fish; they exhibited varying degrees of aggressiveness and did not defend territories. The least aggressive individuals remained either on the bottom or near the surface in front of the downstream screen.

Subordinate fish moved within restricted areas in the pools. When they wandered near the territory of a dominant fish, aggressive encounters in the form of threat displays, chases, or nips often resulted. Occasionally a subordinate fish moved into the cage attached to the downstream screen. The fish would then defend an area near the opening of the cage. Rather than offering the intended refuge, the cage became an easily defendable territory. When the fish was placed back in the channel, it did not return to the cage. The cages were not providing for volitional movement out of the channels and they were removed in subsequent experiments.

Fly larvae were introduced evenly throughout each channel, yet the dominant fish consumed much of the food. In one channel, the fish were fed all the larvae they would eat; in the other, a restricted ration was provided. Restriction of the food supply did not affect consumption by the dominant fish but, at restricted rations, the subordinates consumed less food than at unrestricted rations. In the latter case the dominant fish became satiated and less aggressive, allowing the subordinates a greater food intake. When larvae drifted through the territories of dominant fish, nearby subordinate fish would often approach the territory but would not swim into it to seize a larva. Occasionally a subordinate fish would pick detritus off the rocks ond spit it out when a dominant fish consumed the larva.

Coho held in the aquariums were inactive throughout most of the day. They usually remained motionless near the bottom; the only noticeable activity observed was during the once-a-day feeding. No attempt was made to quantify activity of the aquarium fish, but observed activity differed markedly between the stream and aquarium fish.

Curvilinear regression lines best described the relations between food consumption and growth for both the stream and aquarium fish (Fig. 2). Differences between relations were not statistically significant at the 5% level based on tests of residual sums of squares around individual versus combined regressions; hence, a single curve was fitted to the combined data. Weight change was more variable among starved than fed fish. Rates of weight loss ranged from 4 to 24 mg/g/day.

The tendency of the food and growth relation to decrease in slope beyond 20 mg/g/day of con-

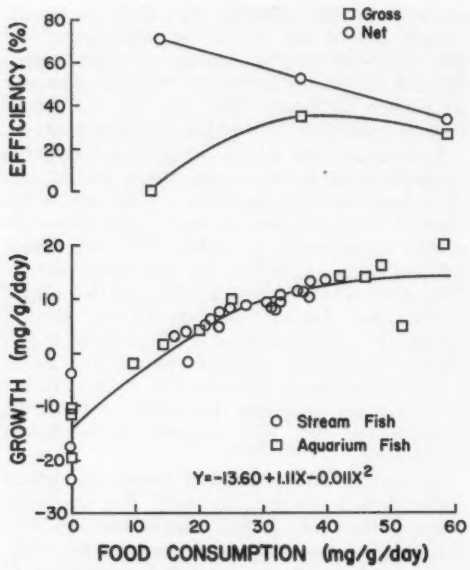

FIG. 2. The relations between food consumption and growth, gross efficiency, and net efficiency for the stream and aquarium fish in Experiment I. R = 0.93, significant at 1% level. The three points plotted on the graphs of gross and net efficiency were selected from the fitted food and growth curve to show the forms of the relations.

sumption implies a decrease in efficiency of food utilization for growth. Gross and net efficiencies calculated directly from the fitted food and growth curve illustrate these changes in efficiency (Fig. 2). As the rate of food consumption increased beyond the maintenance ration, gross efficiency increased until food consumption reached approximately 35/mg/g/day. Beyond this rate gross efficiency declined. Thus, fish with the highest rates of food consumption were less efficient at utilizing their entire ration for growth than were the fish at intermediate rations. Net efficiencies continually declined; each increment of food consumed beyond the maintenance ration resulted in a progressively smaller amount of growth.

EXPERIMENT II

This experiment was designed with the added objective of determining whether cover would have any effect on growth efficiency of the aquarium fish. The number of compartments was doubled by dividing each aquarium in half. Gravel and tunnel-shaped pieces of painted lead (5 x 8 cm) were placed in half of the aquariums to provide cover.

The coho used in this experiment were recently hatched fry obtained from the Oregon Game Commission Research Laboratory in Corvallis. Since these fish were much smaller (Table 1) than fish in the previous experiment, more individuals were used. Riffles in each channel were screened off and five fish were placed in each of the four pools.

Behavior of coho in the stream channels was similar to that of fish in the first experiment, with the dominant fish defending territories and subordinate fish forming hierarchies. Fish in the second experiment exhibited more aggressive and exploratory activity than did the fish of Experiment I (Table 1). The aquarium fish displayed more exploratory activity than did those in the previous trial, yet these fish were still much less active than the stream fish. The fish that had cover available did not appear to use it.

The relations between food consumption and growth rate for the stream fish and both groups of aquarium fish were not significantly different at the 5% level; a single curve was fitted to the combined data (Fig. 3). Providing cover for some of the aquarium fish likewise had no effect on growth efficiency. The fish in this experiment had higher maximum consumption rates than those of Experiment I, yet they showed similar declines in growth efficiency at high rations. This decline in efficiency is indicated by a rapid decrease in the slope of the food and growth relation beyond 45 mg/g/day of consumption (Fig. 3). Again, fish with intermediate ration levels had the highest gross efficiencies.

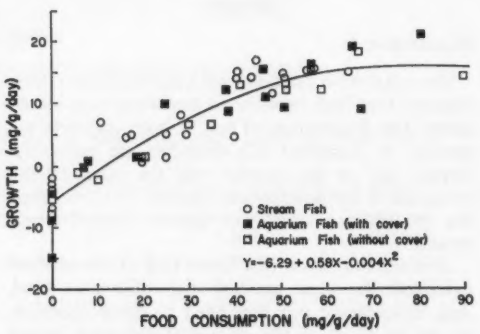

FIG. 3. The relation between food consumption and growth for the stream and aquarium fish in Experiment II. R = 0.93, significant at 1% level.

Experiment III

Failure of the aquarium fish to respond to available cover prompted further changes in design. In this experiment additional overhead cover was provided by placing black plastic containers (15 x 15 x 15 cm) in each aquarium. The bottoms of the aquariums were darkened with sheets of black polyethylene.

As in the previous trial the stream fish were extremely aggressive and exhibited much exploratory activity, whereas the aquarium fish were relatively inactive. There was a difference in locomotory activity between the two groups of aquarium fish. Those fish having cover available hid inside the containers and showed less exploratory behavior than did fish not having cover available.

The growth efficiencies of the stream and aquarium fish were again similar (Fig. 4). The increased cover provided for some aquarium fish had no effect on growth efficiency, although there was a greater degree of variability in the food and growth relation than in Experiment II. The fish in Experiment III had considerably higher maximum consumption rates than those of previous trials. This increase was probably due to higher water temperatures (Table 1). Beyond a consumption rate of 80 mg/g/day there was little increase in growth rate, indicating a sharp decline in gross efficiency. Again, the fish at intermediate rations had the highest efficiencies of food utilization.

Aggression and Growth Efficiency

If aggressive activity were an important energy requirement, we might expect to account for some of the variability in the food consumption and growth relations of fish in the stream tank by considering each fish's level of aggression. Stepwise multiple regression analyses were conducted to assess the effect of aggression on growth efficiency. Growth rate was set as the dependent variable, with food consumption rate and rate of aggressive activity added in order as independent variables. In none of the three experiments did we find a significant reduction in the residual sums of squares when aggressive activity was taken into account. We concluded that the various levels of aggression exhibited by the coho in this study had no effect on their efficiencies of food utilization for growth.

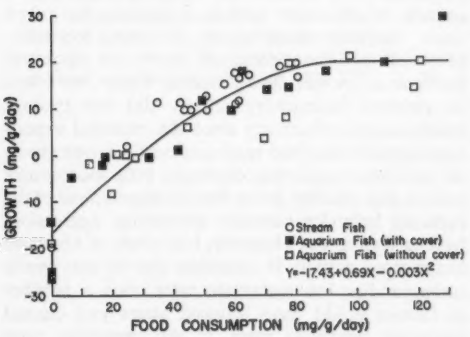

Fig. 4. The relation between food consumption and growth for the stream and aquarium fish in Experiment III. R = 0.93, significant at 1% level.

Discussion

The similarity in growth efficiencies of coho salmon held in the artificial stream and in aquariums suggests to us that growth efficiency data determined from laboratory experiments may provide reliable estimates of food consumption for fish in nature.

Although the experimental stream simulated natural conditions, and the coho exhibited activity patterns typical of those in natural environments, several factors contributed to the artificiality of the system. Perhaps the most important of these was the unnatural feeding regime employed. Kalleberg (1958) demonstrated a diel rhythm in the natural feeding activity of brown trout (*Salmo trutta*). Mason (1969) found diel changes in the levels of aggressive activity of juvenile coho that maintained territories on a riffle in his experimental stream. He showed that the density of fish on riffles increased at dusk and remained high until dawn. This increase in density resulted in high levels of aggression. Mason postulated that the riffles offered superior feeding opportunities. If the invertebrate fauna in Mason's stream channels exhibited high drifting rates at dawn and dusk similar to those found by Waters (1962) and others, then it would seem that juvenile coho were indeed responding to changes in the availability of drift.

Since fish in our study were fed on a regular twice-a-day schedule, their normal diel pattern of activity may have been altered. Certainly they were not stimulated to invade the riffles at night because there were no drifting organisms to attract them. We made a few observations at night; the fish were in the pools and appeared inactive.

Among the salmonids, active night feeding may be unique to juvenile coho. Edmundson et al. (1968) found that juvenile chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*) settled to the bottom and remained inactive at night. They cited other studies that also found salmonids to be inactive at night.

Juvenile chinook salmon in a stream aquarium distributed themselves near food inlet points (Chapman and Bjornn 1969). We introduced the fly larvae into the channels at several different sites so there was no single area that received the most food. The dominant fish avoided the riffles and kept the subordinates behind them in the pools. In this way the dominant fish could seize any larvae that were introduced upstream from them. In most instances the dominants waited for larvae to drift to them rather than making a feeding foray onto the riffles. The resulting distribution of fish in the stream channels may have been influenced by our feeding regime.

The amount of energy coho expend for activity in streams is likely to be dependent upon the current velocity they encounter. Mundie (1969) found that juvenile coho in streams were aggregated in marginal back eddies. When he created back eddies in an artificial stream channel, the number of resident coho in the channel increased. The coho in the main current of the channel defended territories near the bottom where the current velocity was much reduced. Ruggles (1966) also found that stream channels with a pool environment held more coho than did channels consisting of riffles. These studies show that coho tend to avoid positions in a stream where they must swim against a substantial current, thereby minimizing the amount of energy needed for swimming.

In contrast to coho, juvenile chinook salmon seem to favor areas of higher velocity in streams. Everest and Chapman (1972) found a positive correlation between fish size and current velocity. Even though areas of high current velocity in streams probably offer superior feeding opportunities, the fish must expend more energy for swimming there than at stream margins. Thus, swimming could constitute a relatively important energy expenditure for juvenile chinook.

High levels of aggression by juvenile coho in nature could also be an important energy requirement, although this does not seem likely. The lack of sufficient quantitative field data precludes any direct comparison of wild fish with our experimental coho. Chapman (1962) recorded the number of nips made by coho in a natural stream. The rate of aggression based on nips was within the range of our values, but he did not record threat displays so the two results are not directly comparable. The levels of aggressive activity we found were similar to those reported by Mason (1969) from his experimental stream.

The aggressive activity of the juvenile coho in our study had no apparent effect on their utilization of food for growth. However, there must be some point at which aggression can become a major energy expenditure and begin to affect growth rates. From personal observations of juvenile coho in natural streams, we felt that wild fish exhibited less aggression than did those in our experimental stream. Chapman (1966) suggests that there must be a compromise between the area a fish defends and the food supply, whereby the fish can maximize its growth rate through a minimal expenditure of energy for aggression.

In all three of our experiments gross growth efficiency increased from zero at the maintenance ration to a maximum at some intermediate ration and then declined. Others have found a similar relation of growth efficiency and relative ration size when dealing with fish of a restricted size-range (Warren and Davis 1967; Rafail 1968; Brett et al. 1969).

The explanation of declines in growth efficiency at high relative rations is not clear. Warren (1971) suggests that disproportionate changes in either specific dynamic action, activity, or assimilation efficiency with increasing rations could cause declines in growth efficiency. Kerr (1971) postulates that spontaneous activity of aquarium fish increases with ration size, thus causing a decrease in growth efficiency. However, we also found a decline in growth efficiency at high rations in fish held in the experimental stream. Since juvenile coho and many other salmonids in nature seldom attain the high growth rates they are capable of in the laboratory, declining growth efficiency at high rations may not be an important phenomenon. The relatively low growth rates often found in wild populations suggest that their feeding levels correspond to ration sizes within the ascending portion of the growth efficiency food consumption relation.

We did not attempt to measure spontaneous activity of the coho held in aquariums but relied upon frequent observations of their behavior. Most of the coho remained near the aquarium bottoms or moved slowly about. These low levels of observed locomotory activity did not prompt measurements of activity since the intended experimental conditions had been achieved, i.e. one group of individual coho that displayed little locomotory activity and another group that displayed most of the expected behavior patterns: swimming, aggression, feeding forays, etc. However, low levels of observed locomotory activity in aquarium fish do not assure corresponding low metabolic rates since a number of factors could have induced stress and caused increased metabolic rates. If the metabolic rates of the aquarium fish were not raised due to high levels of spontaneous activity or stress, the similar-

ity in growth efficiencies of the stream and aquarium fish with apparent differences in locomotory and aggressive activity might suggest that such activity is not an important energy requirement for juvenile coho in the wild.

We believe these findings indicate that laboratory data can be used to obtain reliable estimates of food consumption for juvenile coho in nature. Future studies of this type should be directed at more closely simulating stream conditions with particular emphasis on a natural feeding regime.

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Clostridium botulinum Control in Cold-Smoked Salmon: a Review

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This review considers questions concerning the growth and toxin production of *Clostridium botulinum* in cold-smoked salmon. In order to produce a product which would be safe as well as acceptable from the consumers' point of view, consideration must be given to the worst abuses to which the product might be exposed. Present knowledge regarding the effects of salt, smoke, and other factors on the preservation of cold-smoked salmon is inconclusive. Strict control of the storage temperature is the only method of positively preventing toxin production or outgrowth of spores of *C. botulinum*. In comparison with fish processed through a hot-smoke procedure, cold-smoked salmon appears to present a lesser hazard, but for unknown reasons.

SOUTHCOTT, B. A., AND W. E. RAZZELL. 1973. *Clostridium botulinum* control in cold-smoked salmon: a review. J. Fish. Res. Board Can. 30: 631–641.

Nous faisons une revue des problèmes associés à la croissance de *Clostridium botulinum* et à la production de toxine par ce microorganisme dans le saumon fumé à froid. Si l'on veut obtenir un produit que le consommateur trouvera à la fois sûr et acceptable, on devra tenir compte des pires conditions auxquelles le poisson peut être exposé. On connaît mal à l'heure actuelle les effets du sel, de la fumée et autres facteurs sur la conservation du saumon fumé à froid. Un contrôle serré de la température d'entreposage est le seul moyen sûr de prévenir la production de toxine et la croissance trop rapide de spores de *C. botulinum*. Comparativement aux poissons soumis au procédé de fumage à chaud, le saumon fumé à froid semble présenter moins de danger, pour des raisons que nous ignorons.

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THERE is a paucity of information regarding the microbiology of mild-cure salmon. The fact that this product is consumed without heating after purchase necessitates that caution be exercised at all stages of its production, particularly with respect to control of the ubiquitous, spore-forming, toxigenic bacterium *Clostridium botulium*. Although the problem may be thought to be of small consequence because of the relatively small consumption of cured fish in Canada, the serious nature of an outbreak of botulism would have a very deleterious effect on both domestic and export markets for a specialty product which is more valuable per unit than most other fishery products. This review assesses the literature pertaining to the smoking process in general, and its applicability to the mild-cure treatment of salmon in particular.

In the disposition of the world catch of fish over the 5-year period 1965–1969, the quantities used for marketing fresh and for curing have remained constant, while those frozen and canned have been steadily rising, as has the total world catch (FAO 1971). Relevant figures for Canadian fish consumption in 1954 and 1969 (Anon. 1971) are:

	1954	*1969*
Per capita consumption of all fish (*lb*)	13.5	12.1
" " " " cured* fish (*lb*)	1.9	1.0
% of total consumption provided by cured products	14.1	8.3
% of total catch used for curing	30.0	11.0

(*cured includes smoked, salted, pickled)

However, the value per unit of smoked salmon exports from Canada is much greater than that of the total smoked fish export or the total fish export (Anon. 1971):

	1969			*1970*	
	Q	V	V/Q	Q	V
Total fish exports	760,000	247,000	0.33		280,022
Total smoked fish exports	10,958	2,629	0.24	7,882	2,859

Printed in Canada (J2739)

	1950			1970	
	Q	V	V/Q	Q	V
Smoked salmon exports	450	703	1.56	659	1,235
Mild-cure salmon produced, Pacific area	231	367	1.58	395	704

Q = quantities in thousands of pounds
V = values in thousands of dollars
V/Q = " " dollars per pound

The largest importers of smoked salmon from Canada are South Africa, Australia, Hong Kong and Sweden.

Castell (1964), in a report on the significance of botulism in the Canadian fisheries, emphasized that every outbreak of botulism from fishery products in Canada has had a history of fish which had been poorly processed or grossly mishandled. Castell did not discuss the processing conditions necessary to ensure safety of smoked products in the event of any mishandling.

The temperature attained during the cold-smoking of salmon (29 C=84.2 F; FAO 1970) is not high enough to destroy spores or toxins of *C. botulinum* A, B, or E by thermal action alone; if such destruction is a significant result of the process, it must depend upon the combination of conditions prevailing in the fish flesh and in the smoking chamber. Most of the work reviewed in the following report has employed hot-smoked whitefish as the substrate, because this has been the vehicle in recent outbreaks. Hence, conclusions reached by various workers with regard to the influence of salt, smoke, moisture, *p*H, and temperature on *C. botulinum* spores in hot-smoked whitefish or in broth cultures cannot necessarily be applied to cold-smoked, or mild-cure, salmon. Processing methods for mild-cure salmon vary widely between processors and are not government regulated with regard to temperature of operation or salt and moisture content of the finished product.

Smoked salmon products are traditionally considered to be relatively safe, but for unknown reasons. Lewis and Hall (1970) stated that examination of fish processing plants and the results of recent research have revealed a great need for proper sanitation practices and more rigidly controlled heating and smoking procedures.

In any investigation of the possible food-poisoning hazard posed by mild-cure salmon or other salted smoked fish, consideration must be given to the worst abuses to which the product might be exposed at the processing, distributing, and consuming levels.

Geographical Distribution of *Clostridium botulinum*

It must be considered possible, although not probable, that a fishery product may be contaminated with spores of any known type of *C. botulinum* A–F. All types have been isolated from various freshwater and marine sources, and the tendency to consider type E as a primarily marine type has been replaced by an awareness of the wide distribution of all types. Riemann (1969) has stated that "...type E has in the past been associated with water and marine environments, whereas A, B, C, etc., have been regarded as terrestial types. More recent studies have shown, however, that types A and B may be present and even outnumber type E in marine environments."

Johannsen (1963) reported that type E of terrestrial origin was ubiquitous in Sweden and adjacent waters. Cann et al. (1965, 1966, 1967, 1968) studied the distribution of *C. botulinum* types in Britain and in Scandinavia and found that the Baltic Sea and Scandinavian waters had pockets heavily contaminated with type E, although in the North Sea and waters around the British Isles, no type E but, rather, a nonproteolytic type B was present. Many samples of toxic herring were reported in Britain; these all contained type E toxin and had all been imported from Scandinavia, fresh or vacuum-packed. Cann stated that there was little danger from products which would be cooked before being eaten, but that "...the main danger lies in products such as smoked salmon which are eaten without further cooking. It is essential that all fish products, whether vacuum-packed or not, should be stored below 4 C or preferably frozen from the time they are processed until eaten, and all packs should be so labelled." The nonproteolytic type B found in Britain was felt to be as potentially dangerous as type E because of its properties of growth and toxin production at low temperatures. Abrahamsson (1967) found type E toxin in an enrichment culture of smoked Baltic eel. Baumgart (1971) reported fatal type E cases in Germany from frozen eviscerated trout and smoked trout fillets from the same source. Nielsen and Pedersen (1967) isolated type B from 1.68% of the smoked Baltic salmon examined.

Isolations of all types, A–F, have been made from sea water, mud, fish, and shellfish from the east and west coasts of North America, from the Great Lakes, and from the Gulf of Mexico (Bott et al. 1966, 1968; Carroll et al. 1966; Chapman and Naylor 1966; Craig et al. 1968; Eklund and Poysky 1967, 1970; Hayes et al. 1970; Kautter 1964; Laycock and Loring 1972; Laycock and Longard 1972; Segner et al. 1971; Pace et al. 1967. Presnell et al. 1967; Ward et al. 1965, 1967; Williams-Walls 1968). Boyd and Southcott (unpublished data) found types A, B, and E in the lakes of central Canada. The distribution of spores of *C. botulinum* throughout the world has been further discussed by Sakaguchi (1969) and Riemann (1969). In view of these and other reports it is apparent that the presence or absence of any strain of *C. botulinum* on a fish sample is the consequence of random sampling. (The occurrence of a positive sample from fish which are to be smoked

is therefore immaterial, since negative samples also occur essentially by chance.) It would therefore seem appropriate to consider the adequacy of processes employed in the preparation of smoked products such as mild-cure salmon, both to kill spores during smoking and to prevent germination and toxin formation by spores introduced subsequently.

Effect of Salt on *Clostridium botulinum*

Although salt contributes to the quality of smoked fish by firming the flesh, preventing discoloration and imparting some flavor, it does not appear to prevent bacterial growth or spoilage at concentrations which would be acceptable to most consumers (Weckel and Wosje 1966). An FAO report on smoke curing of fish (1970) stated that presmoking salt treatment, by use of brine or solid salt, in certain circumstances inhibits bacterial action; but the report stated that the 2–3% salt in most modern products has only a slight effect on the bacteria which spoil fish. In spite of the fact that attaining a uniform concentration of salt in the water phase of the smoked product is considered important in reducing the potential hazard of growth of *C. botulinum*, studies by Weckel and Wosje (1966) using Great Lakes chub showed that it is difficult to achieve uniform salt content among large numbers of fish and between different parts of the same fish brined prior to smoking. The age and size of fish, the method of capture, and the degree of scaling occurring during transfer of fish from boat to plant were found to influence salt uptake. Weckel and Wosje reported that fish aged 6 days in water at 4.4 C prior to being brined contained more salt than fish brined fresh; salt uptake was greatest in fish which had been frozen before brining. The percent salt in both the edible flesh and the water phase of the edible flesh was greater in medium-sized fish than in large fish. Fish in rigor absorbed less salt than post-rigor fish; scaled fish absorbed more salt than did unscaled fish.

Other significant factors affecting uniformity of salt content of brined fish were, predictably: the concentration of salt in the brine and the maintenance of the concentration during the brining procedure, temperature of the brine, length of brining time, and ratio of weight of brine to fish. More salt was absorbed by fish brined in a greater concentration of salt; a brine in which the concentration of salt was maintained during the brining period resulted in higher salt concentration in fish in a shorter time than a brine of decreasing salt content. Weckel and Wosje demonstrated that an increased ratio of weight of brine to weight of fish (from 2:1 to 5:1) raised the concentration of salt in the total edible flesh, especially during the latter stages of the brining period. During equilibration, the loss of moisture from fish to brine caused less relative reduction in concentration of salt in the brine when greater brine to fish ratios were used.

Ingram and Kitchell (1967), in a review of the action of salt as a food preservative, have noted the differences in the action of salt under the influence of auxiliary factors such as temperature and *p*H. The limiting salt concentrations for growth of various bacteria depend on the nature of the medium or the tissue under study; quoted figures for salt tolerance serve as no more than a comparative guide. These authors state that although the lethal action of salt against bacteria is less at low temperatures, the influence of temperature on the inhibitory (as distinct from lethal) action of salt is less clear; in spore-forming bacteria, the greatest salt tolerance exists at the optimum temperature for growth.

Bain et al. (1958) stated that the flora of unused fresh brine is conditioned largely by the flora of the salt used; immediately after the first lot of fish has been brined, however, there is a large increase in bacterial load, and this increase continues as more fish are brined. Subsequently, the major influence on brine flora is the flora of the fish being processed.

Liston and Shewan (1958) reported that 85–90% of the flora of such fish as haddock and cod survived much longer than the normal brining time for such fish in 20% (w/v) salt. These authors concluded that light brining generally has no marked effect on the numbers of bacteria present on the fish; qualitatively, the gram-positive types (micrococci and coryneforms) increase in importance at the expense of the gram-negative group (especially *Pseudomonas* and *Flavobacterium* spp.). No anaerobic investigations were carried out by Bain or Liston.

Of more direct interest, Pedersen (1958) reported the complete survival of spores of different types of *C. botulinum* in curing brines at 10 C having 15–22.4% NaCl. Some of the brines contained nitrate; *p*H values were 4.8–7.9.

Effect of Smoke on *Clostridium botulinum*

Intense combustion of wood results in the breakdown of intermediate organic substances into the final combustion products, carbon dioxide and water, and the smoke essential for a curing process will not be formed. Incomplete combustion results in production of smoke containing organic substances which impart taste, aroma, and color

to smoked fish. The output of organic substances can be controlled by varying the pyrolysis temperature of the wood and the amount of air admitted to the hearth.

General studies on smoke formation have not been directly concerned with the antibacterial effects of smoke components; however, the combination of salting, heating, and smoke deposition on fish is generally effective in retarding spoilage.

There are two distinct types of smoking process, differing in the amount of heat to which the fish is subjected. In cold-smoking, the temperature of the fish should not exceed that at which the protein is denatured, 29 C (84.2 F), and the fish remains uncooked. In hot-smoking, the fish are intentionally cooked in the smoke; the temperature in the kiln is raised quickly to 100 C, during which time the fish temperature reaches 65–75 C (149–167 F) depending on the requirements of the product.

Smoking was formerly associated with heavy salting resulting in a "hard" cure; the present trend is toward more lightly processed products.

Properties, Production, and Deposition of Smoke; Use of Liquid Smoke

Wood smoke is a typical aerosol; the solid (smoke proper) and liquid (vapor or mist) particles are dispersed in a gaseous medium (the gas mixture produced by the burning wood). Cutting (1965) has discussed the breakdown of wood lignins and the resulting phenolic constituents of smoke; these groups are present, though in greatly different relative concentrations, in both the particle and vapor phases of wood smoke, the vapor phase being much richer in volatile phenols than the particle phase. Brownian movement of the particles results in formation of aggregates which, in the case of fish smoking, are deposited on fish surfaces. A high relative humidity (RH) in the kiln increases the rate of aggregation and results in rapid deposition of particles on the fish; rapid mixing of air with newly-formed smoke causes dispersion of particles, delayed aggregation, and slower deposition on fish surfaces (Zaitsev et al. 1969). The quantity of smoke deposited is a complex function of the composition and concentration of the smoke, the environmental conditions, and the nature of the surface (Cutting 1965).

The process of deposition of smoke on wet surfaces, such as fish, has been found to be completely different from that on dry surfaces; in the former case, the vapors contribute more to the total deposit than do the particles. The rate of deposition of smoke increased as the rate of drying of fish during smoking increased; the fact that this relationship was the same whether particles were present or not, and that the phenol distribution patterns were the same in fish smoked in total smoke or in vapors, led to the conclusion that the particle phase of wood smoke played an insignificant role in the smoking process (Cutting 1965). The FAO report of 1970 stated that the tarry droplets (particles) in smoke are not an essential part of the smoking procedure.

Depending upon the conditions of smoke generation, the smoke and fish cured in it may contain varying amounts of the carcinogen 3,4–benzopyrene; when the combustion temperature rises, the content of 3,4–benzopyrene in the tar fraction of the smoke increases. The electrostatic precipitation of the tar from the smoke eliminates this carcinogen (Zaitsev et al. 1969). Zaitsev reports that cold-smoking, because of the low temperature employed, results in much lower 3,4–benzopyrene content in the smoke and in the fish than does hot-smoking. Experiments are being carried out in the USSR to develop a carcinogen-free smoking liquid to take the place of normally produced smoke (Foster 1959; Zaitsev et al. 1969).

Handford and Gibbs (1964) suggested that liquid smoke or smoke condensates could possibly offer an attractive alternative to the conventional smoking process; some of the variables associated with the traditional smoking process could be reduced. The composition of liquid smokes, usually compounded to impart good flavor, may not be compatible with maximum antibacterial activity.

Cutting (1965) has noted that, in many countries, the use of smoke concentrates would pose difficulties in the matter of compliance with food laws. Evidence would have to be produced to show that the composition of the final product was not significantly different from that customarily produced by deposition on fish of components from the vapor fraction of normal smoke. Cutting reports that the appearance and flavor of experimentally dipped products are noticeably different from those of customarily accepted products.

Zaitsev et al. (1969) has reported that in the USSR, some fish plants employ a combination of dipping and smoking which shortens process time and reduces losses. However, when judged by a taste panel, smoked fish produced in this way are inferior to the normally smoked product. Fish smoked in the combined way had good keeping qualities.

Antibacterial Action of Smoke Components

Opinions regarding the antibacterial effects of smoke constituents present on fish vary considerably

because differences in experimental conditions or lack of experimental detail render exact comparisons of detail impossible.

Shewan (1949) reported that although many chemical compounds with bactericidal or bacteriostatic properties were present in smoked fish, little was known of actual amounts absorbed. Due to the absorption of acid constituents from smoke, the *p*H of fish flesh falls during smoking; the main effect of this increase in acidity is to render the microorganisms on the fish more susceptible to the bacteriostatic and bactericidal action of the smoke. Shewan found that the cold-smoking process resulted in a considerable kill-off of the bacterial load on the fish, and concluded that because of the temperature characteristics of the cold-smoking process, it was the smoking *per se* which effected the kill-off. The smoked fish had increased percentages of micrococci but lower percentages of asporogenous rods. Shewan noted that in stale fish, bacteria had penetrated the flesh, particularly by way of the vascular system near the backbone, and that such bacteria might be protected from the destructive action of smoke.

Shewan (1961) decided that the phenolic constituents of smoke are the most active bactericidally, and not the formaldehyde as previously thought, although work reported from his laboratory had been concerned with the effect of mixed smoke fractions against fish spoilage bacteria, and no anaerobic spore-formers were tested.

In contrast, Nielsen and Pedersen (1967) studied the inhibitory effect of smoke constituents on *C. botulinum* types B and E in smoked salmon ("Danish", presumably cold-smoked) and reported anew that the inibitory effect was related to the formaldehyde content, not to phenols or oxidized fatty acids. Smoked salmon samples had minimum formaldehyde values of 20–40 μg/g. In broth studies, 35–40 μg formaldehyde per ml completely inhibited germination of type B and E spores for more than ten days. In fresh salmon, Neilsen found that even small numbers of spores could germinate and produce toxin; moderate numbers of spores resulted in high levels of toxin in the fish. Type E produced 3000–6000 MLD (mouse minimal lethal dose) per gram and type B produced 30,000 MLD per gram. The work of Nielsen and Pedersen differed from that previously reported in that smoked salmon and *C. botulinum* were employed; the differences of opinion regarding the antibacterial constituents of smoke cannot, therefore, be readily evaluated.

Zaitsev et al. (1969) reported, without source references, that the bactericidal effect of smoke is conditioned not only by its formaldehyde content but also by phenol, organic acids, and other compounds. Zaitsev et al. stated that pure cultures of non spore-bearers will be killed by smoke after 3 hr, whereas spores of certain *Bacillus* spp. will survive in thick smoke for 7 hr. Results of studies on the effect of smoke on fish showed that smoke with a temperature below 40 C (104 F) had weak antiseptic action and that only with hot-smoking was the number of bacteria substantially reduced due to the high temperature of the smoke (Zaitsev et al. 1969).

Since fish has a bacterial population, initial and introduced during handling, that is variable, and since the compounds which kill the greater part of the original flora way well be different from those which restrict the growth of smoke-resistant organisms, it seems reasonable that the antiseptic compounds of smoke should be defined only in terms of the bacterial population and the nature of the smoked product (Cutting 1965).

Quantitation of Smoke Components

Various investigators have studied methods for the estimation of smoke components in smoked fish. However, it has not always been explicitly stated what property of the final smoked product was being affected by a change in the level of the test substance.

Cutting (1965) has discussed methods used by various workers in the period 1927–1958 for estimation in fish of formaldehyde, volatile acids, acetone, and phenol. Although these methods were sensitive and accurate, they provided information about the deposition of a relatively small proportion of the total composition of wood smoke and should therefore be used only as an index when comparing products where conditions of smoke production are the same.

Zaitsev et al. (1969) reports that, until 1960, the degree of smoking was estimted in the USSR by total phenol content in the finished product. Recently, the composition of the phenol mixture has been estimated by chromatographic distribution.

In most of the studies reviewed by these authors, few correlations were made between antibacterial effects and quantified constituents of smokes. No specific data were obtained in repect of *C. botulinum*. Thus, it is presently impossible to utilize chemical tests to measure microbiological safety.

Furthermore, such emphasis on the smoking process *per se* should be considered in the light of the fact that cold-smoked salmon is stored and consumed without further processing. Even if some decrease in *C. botulinum* numbers had been shown, the problem would remain to correlate smoke constituents with suppression of the organism during subsequent storage.

Combined Effects of Salt, Moisture, *p*H, Temperature, and Smoke on *Clostridium botulinum*

It is obvious that, in the production of smoked fish products, the maintenance of proper sanitary conditions during processing and the application of the proper combination of brining, smoking, and cold storage must be relied upon to destroy some of the bacterial spores present and/or to prevent germination, outgrowth, and toxin production by the survivors.

On Outgrowth of *C. botulinum* Spores

We have pointed out previously that the handling of cold-smoked salmon could favor the production of toxin by *C. botulinum* which survived the smoking process, unless the smoke also conferred residual bacteriostatic properties. Presumably, the combined effects of various elements of the smoking process might create a residual effect, whereas singly they would not.

Segner et al. (1966), in a study of the inter-relationships of temperature, *p*H, and salt concentration on the outgrowth of *C. botulinum* type E, reported that type E spores possess a level of salt tolerance considerably below that recognized for types A and B. Segner also noted that in trypticase-peptone-glucose broth, salt concentrations not sporistatic to type E at its optimum temperature could nevertheless cause a significant extension of outgrowth time at low temperatures. At 16 C, 21 C, and 30 C, three type E strains required 5% salt for inhibition; at 8 C and 10 C, 4.5% salt was inhibitory. Segner stated that there was a marked interaction of *p*H and low temperature in the delay of type E growth in broth; fish homogenates did not show this interaction, consequently Segner advised that growth and toxin production of type E should be suspected in foods at low temperatures at *p*H levels normally permitting growth at 30 C.

Emodi and Lechowich (1969) found that salt tolerance of type E strains was lower at *p*H 6.5 (as in smoked fish) than at *p*H 7.2. Salt at 4.87% inhibited all strains tested, in close agreement with the work of Ohye et al. (1967) on type E. In addition, the bacteria were more susceptible to the combined inhibitory effects of salt and acid when the temperature was raised.

On Toxin Production by *C. botulinum*

The most important result of the entire smoking process is the prevention of toxin production in the product before it is consumed. Whether this result is achieved through sterilization of the fish, through inhibition of outgrowth of organisms surviving the smoking process, through interference with toxin production by outgrowing organisms, or by inactivation of toxin once produced, is of lesser significance. Once again, data relevant to the cold-smoking of salmon are scanty.

Sheneman (1965) studied *C. botulinum* type E toxin formation in ground, commercially hot-smoked whitefish. An 18-hr brining in 7.7% salt followed by smoking at 82.5 C (180 F) for 30 min gave a product of 5.5% brine concentration which was unable to support the growth of 11 strains of type E. When the product brine concentration was 3%, toxin was produced. Thus, a definite salt content was essential, even in a hot-smoke process. Sheneman also inoculated ground raw whitefish with 11 strains of type E and heated the samples to various temperatures; it was found that all 11 strains produced high levels of toxin in the raw fish and that three strains still produced toxin after heating to 82.5 C (180 F) for 30 min. No strain survived 88 C (190 F) for 30 min. Therefore, the salt and smoke in combination lower the temperature required to prevent toxin formation.

Abrahamsson et al. (1966) reported toxin production by *C. botulinum* type E between 3–30 C in fish dialysate and chopped meat medium. Data in agreement with those of Segner et al. (1966) on outgrowth demonstrated that the lowest concentration of salt to inhibit toxin production in broth varied with the temperature and duration of storage. Type E spores heated in minced raw herring survived 110 min at 80 C or 10 min at 90 C and produced toxin after long storage at 20 C. Type E toxin was more thermostable in meat or fish dialysate than in buffer.

Cann et al. (1967a) tested four commercially available fish products, representative of those on sale in Britain, for their ability to support toxin formation by type E at various times and temperatures. Fresh herring and halibut were highly toxigenic; kipper (smoked herring, 3.2% salt) was slightly toxigenic; toxin production in smoked salmon (presumably cold-smoked) was slow and never reached high titres. However, Cann et al. considered the greatest danger was likely to occur with smoked salmon, which is eaten without further cooking; he concluded that all types of packaged fish commercially produced should be kept below 4 C from time of processing until sold to the consumer.

Ohye and Christian (1967) and Ohye et al. (1967) studied the effects of temperature, *p*H and a_W*

*a_W = water activity, i.e. $\frac{\text{vapor pressure of the solution}}{\text{vapor pressure of pure solvent}}$

on growth and toxin production by *C. botulinum* types A, B, and E. For type E, the optimum a_W for growth was found to be approximately 0.995 at all temperatures; growth rate declined rapidly at a_W below 0.990. At optimum conditions of temperature and *p*H in broth, the minimum a_W supporting growth were: type A, 0.95 (=8% salt); type B, 0.94 (=9.4% salt); type E, 0.97 (=5.1% salt). In the absence of effective smoking treatment, 10% salt (a_W 0.938) could be considered marginal for types A and B at high temperature and optimum *p*H. Reduction of temperature markedly increased the water activity requirements for spore outgrowth. Toxin was formed by all types of *C. botulinum* tested at the lowest a_W supporting growth at each *p*H and temperature; Ohye et al. (1967) suggest that a borderline area may exist at low a_W where growth might occur without toxin formation, but that this area would be narrow and of no practical significance.

Boyd and Southcott (1971) tested three strains of type E in homogenates of fresh frozen cod and found considerable variation in the brine concentration permitting growth and toxin production by different strains. Toxin was produced in homogenates of 4.4% brine concentration by the Tennessee strain; the Minnesota strain grew but did not produce toxin in 5.5% brine concentration.

On Viability of *C. botulinum*

Some work on survival of *C. botulinum* through phases of the hot-smoking process should be noted, although the results require reevaluation under cold-smoking conditions.

Christiansen et al. (1968) injected six strains of type E spores into whitefish before hot-smoking (82.5 C × 30 min) and found that the smoked fish was not free of viable spores; no toxin was formed with a brine concentration of over 3% in the loin muscle. Moisture in the atmosphere did not reduce the incidence of fish with type E survivors.

Pace et al. (1972) has reported that the heat destruction, in the absence of smoke, of *C. botulinum* spores (type B and E) on whitefish chubs (*Leucichthys* spp.) was dependent on the RH of the chamber in which the fish were heated. Fish brined under commercial conditions (tissue brine 1.5–3%) had type E spore populations reduced 5 logarithms or more by an internal temperature of 82 C (179.6 F) for 30 min at 70% RH; heating at 88 C (190.4 F) and 70% RH reduced the spore population more than 6 logarithms. A 5–6 logarithm reduction was also observed when fish inoculated with type B spores were processed at 82 C (179.6 F) in 70% RH. In contrast, no appreciable destruction of type A spores occurred on fish processed 30 min at 88 C (190.4 F) with RH of 30–90%.

Alderman et al. (1972) have recently reported on the survival of type E through the fish "smoking" process. Chub were inoculated and than heated (unbrined, unsmoked) at 82.5 C (180 F) for 30 min (hot-smoking process). The percentage of such fish containing viable type E spores was significantly lower among fish heated in an environment of high moisture than among those heated in an atmosphere of low moisture. Alderman et al. also suggested a source of the cross-contamination often noted in fish smoking tests: as drippings containing spores dry during early stages of smoking, the spores could become air-borne and be circulated in the smoke chamber. Spores heated in raw fish had greater heat resistance than those heated in precooked fish; possibly the proteins in the raw substrate coagulate and coat the spores, resulting in a microenvironment of low water activity in which they are better able to withstand heat.

Cold-Smoking of Salmon and Storage of the Finished Product — Implications of the Available Information

Cold-smoked salmon is eaten uncooked; since the smoking process encompasses so many variables affecting survival of bacterial spores, and since both packaging and handling may contribute to contamination or proliferation of organisms after the smoking process, only strict control of storage temperature can be relied upon to prevent formation of toxin in the product. It is unlikely that strict temperature regimes have actually been followed in the recent past, and yet no outbreaks of botulism have been reported. The fact that so much concern has been expressed regarding the possibility of botulism from hot-smoked whitefish can be taken to mean only that cold-smoked salmon in some unknown way represents a much lower hazard.

Thatcher et al. (1962) suggested that the anaerobic packaging of foods formerly marketed in a manner that provided ready access of air may increase the hazard of food poisoning by allowing toxin production by *C. botulinum* and staphylococci while minimizing the activities of common spoilage organisms whose offensive changes could give warning of potentially unsafe conditions; this may be particularly pertinent to the production of toxin by *C. botulinum* type E, since this organism can elaborate toxin at refrigerator temperatures. It seems apparent that cold-smoked products

would be at great risk should they be vacuum-packed, unless extreme care was exercised regarding storage temperatures.

Pace et al. (1967), reporting on smoked whitefish in the Milwaukee area, concluded that "smoked whitefish chubs processed at 82.2 C (180 F) for 30 min must be considered not only as highly perishable but also as potentially dangerous. To further assure consumer safety, fish processed under these conditions should be limited to a shelf life not to exceed 7 days at 4.4 C (40 F) or below."

Hayes et al. (1970) reporting on a survey for the presence of spores of Type E in smoked fish products of the Pacific Northwest in which culture filtrates of 4.6% of the samples contained type E toxin, has stated that "... the extreme variations in moisture content found among the various samples of these smoked products is evident and apparently reflects the lack of any serious effort on the part of small processors to control this factor. Similar wide variations are apparent in the salt content found in these products ... such products could constitute a hazard to human health, given the right environmental conditions for storage."

A determination of the salt content of a sample would be of no value in itself since levels in any acceptable product would not be high enough to control *C. botulinum* outgrowth and toxin production; in addition, uniformity of salt distribution is very difficult to achieve.

The influence of various smoke components has not been firmly enough established to allow a test for phenol or formaldehyde to be the criterion which would indicate that a product had been properly smoked. It has not been demonstrated that there is a universal parallel between any one smoke component and microbiological quality, let alone toxin production during storage.

The fact that, at the end of the cold-smoking process, salt and smoke will have contributed in a nonspecific way to the stability of a product possibly containing an unknown number of *C. botulinum* spores, demands that stringent measures be adopted immediately after processing to prevent outgrowth and toxin production by such spores.

Schmidt et al. (1961), using a beef stew substrate, showed that a storage temperature of 3.3 C (38 F) was not safe over an extended period with respect to preventing outgrowth and toxin production by spore inocula of *C. botulinum* type E. Comparison of results at 3.3 C (38 F) and 2.2 C (36 F), however, suggested a very sharply defined critical temperature zone above wich spores of type E could present a public health hazard during moderately long storage periods.

The minimum growth temperature for types A and B is generally accepted to be 10 C (50 F). Eklund et al. (1967) demonstrated outgrowth and toxin production by a nonproteolytic strain of type B after long incubation at 3.3 C (38 F) and after a shorter time at 4.4 C (40 F) and 5.6 C (42 F).

Walls (1967) reported that the Langeland (proteolytic) type F was capable of growth and toxin production at 4 C (39.2 F). The nonproteolytic type F (Eklund et al. 1967) outgrew and produced toxin at 3.3 C (38 F) but not at 2.2 C (36 F).

Shewan and Cann (1965) in discussing botulism in smoked salmon stated that the concentration of salt is not usually high enough to prevent growth altogether, and that since neither the smoking nor the drying are particularly severe, type E *C. botulinum* can survive and flourish in the finished product unless it is kept cool. Shewan recommends that the product be cooled immediately after processing to 2.8 C (37 F) or below and kept cool until eaten.

Cann et al. (1966) stated that smoked salmon, whether vacuum packed or not, should be stored below 4 C (39.2 F), or preferably frozen until eaten.

The FAO report (1970) on smoke curing of fish gives the storage life of cold-smoked salmon fillets as follows:

at 16 C (60 F)	2–3 days in first-class condition 4–5 days in edible condition
at 0 C (32 F)	4 days in first-class condition 10 days in edible condition

In the same report, the following figures are given for frozen storage of smoked fatty fish — no processing details are listed, nor are species of fish stated:

at –9.5 C (15 F)	3 weeks in good condition; at 2 months inedible
at –20 C (–5 F)	2 months in good condition; at 5 months inedible
at –29 C (–20 F)	4½ months in good condition; at 9 months inedible

Georgala and Hurst (1963) reported that the spores and toxin of *C. botulinum* are unaffected by freezing; thus, if a frozen product containing type E spores were to be thawed and stored above 3 C (37.4 F), the spores could possibly outgrow and produce toxin. Any toxin formed previous to freezing would remain through freezing and thawing.

Taken together, these reports indicate that outgrowth and toxin production in several media are related to the storage temperature of fish products.

If the incalculable preservative effect of smoke is disregarded, the storage temperatures recommended by some authors would be too high with respect to type E, and nonproteolytic type B and type F. Strict safety considerations dictate a storage temperature of 2.2 C (36 F) or less. It is almost certain that this is seldom achieved.

Conclusions

It is impossible to ignore the continuing uncertainties about the limits which should be placed on the many variables in the hot-smoking process. It is even more difficult to extrapolate to the cold-smoking process for salmon because of the lack of data on which to base comparisons. We must conclude that the legitimate concern expressed for the safety of poorly refrigerated hot-smoked products — Pace et al. (1967, 1972) — should be applied even more forcefully to cold-smoked salmon. This is not because the latter has caused problems, but because there is no process variable nor combination of variables which when controlled would yield a product that could be abused with impunity.

It appears that the hot-smoke process is successful because of the combination of processing procedures, each of which is only partially effective alone. For each, the following limits seem reasonable:

Salt: the concentration in the final product should be uniform, but in all samples should exceed 4.7%.

Moisture Content: the disadvantage of moisture above an a_W of 0.94 in the flesh during subsequent storage must be balanced against the better killing effect of moist heat (RH above 70%) during the smoking process. So few comparisons have been made on this basis that an ideal moisture level cannot be specified. More experimentation is required.

Smoke: there is no chemical method of defining adequate smoking results. In the smoking process, the temperature should reach 82.5 C (180 F) in the fish and be maintained for at least 30 min. As noted above, the RH of the chamber should be not less than 70%.

Acidity: the smoking process should lower the *p*H of the fish flesh to 6.5 or less.

Nevertheless, no laboratory studies using fish inoculated with the more resistant strains of *C. botulinum* (types A and B) have been performed under the above set of conditions to determine effectiveness in killing the organism, nor in preventing toxin production during subsequent storage at various temperatures; nor has the maximum storage temperature been assessed which prevents toxin production by spores of various types introduced subsequent to the smoking process. It is to be hoped that the definitive experiments will soon be done.

The situation with respect to cold-smoking salmon is even more nebulous; the commercial process is poorly defined and varies greatly from one locale to another. It is likely that salt concentrations in the flesh of 1.5–3% are dangerously low (even when they are attained). The long smoking time at low temperatures (one week or less at 29 C (84.2 F) or less) gives no assurance whatever of killing spores, nor even inactivating preformed toxin if it should be present. The variation in technique undoubtedly yields products of greatly varying moisture content. No data on acidity of cold-smoked salmon flesh are available. Although storage of the product at 2.2 C (36 F) is obviously prudent under these circumstances, it is unlikely that it has been practised in the past nor can it be achieved routinely in the future.

Until the bacteriology of the cold-smoked salmon process is under more precise control, both the producers and distributors of this product bear a heavy share of responsibility for its safety. Distributors should recognize the refrigeration requirements; producers should take pains to maintain sanitary practices in their operations. These would include the use of easily cleaned materials of construction and routine cleaning and disinfection of their equipment.

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Uptake of Mercury by Caged Rainbow Trout (*Salmo gairdneri*) in the South Saskatchewan River

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Rainbow trout (*Salmo gairdneri*) were held in cages in the South Saskatchewan River to assess mercury uptake following curtailment of mercury discharges to the river. There was a rapid uptake of mercury by the fish during the first warm summer period (1970) with much less uptake during the rest of the year. A second summer experiment (1971) produced no significantly different results suggesting that a long-term contamination exists within this river system. Analyses showed that the bulk of mercury present in the fish carcasses was present as methylmercury.

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Des truites arc-en-ciel (*Salmo gairdneri*) ont été maintenues dans des cages dans la Saskatchewan-Sud, dans le but de mesurer la captation de mercure après réduction des déversements de mercure dans la rivière. Il y eut rapide absorption de mercure par les poissons durant la première période chaude de l'été (1970) et diminution prononcée le reste de l'année. Les essais d'un deuxième été (1971) ne produisirent pas de résultats très différents de ceux du premier été, ce qui suggère une contamination à long terme dans le bassin de cette rivière. Des analyses ont démontré que la majeure partie du mercure présent dans la carcasse du poisson s'y trouvait sous forme de mercure-méthyle.

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IN early 1970, following the discovery of mercury-pollution in the Saskatchewan River System (Wobeser et al. 1970), cages containing rainbow trout (*Salmo gairdneri*) were placed in the river at sites above and below the suspected sources of mercury entrance into the river, the Saskatoon sewer outfall and an outfall from a chemical complex located about 500 m downstream of the sewer outfall. Hasselrot (1964) studied pulp mill effluents by the use of caged fish and (Hasselrot 1968) used caged fish to study mercury pollution in Swedish waters. The present series of experiments were designed to give information on the rate of mobilization of mercury into fish under natural conditions and the extent of the damage from these discharges, and some estimate of how long the mercury present in bottom sediments downstream of these outfalls would continue to contaminate fish.

Materials and Methods

Cages (37.5 × 37.5 × 75.0 cm) were constructed out of ¾-inch fir plywood with a large stainless steel mesh (1-cm) door and stainless steel mesh vents (10 × 60 cm) along the sides. Each cage was stocked with approximately 75 1-year-old fish (11.5–15.4 cm; Drew Hatchery, Spokane, Wash., USA). Immediately prior to stocking, six fish were taken for analysis. At each sampling period (2-week intervals) six fish were taken at random and pooled homogenates were prepared from the following tissue: liver, kidney, and the headless, dressed carcasses. The homogenates were stored at –40 C until analysis and

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unthawed portions were taken for analysis. In a few select cases analyses were carried out on tissue homogenates from individual specimens to determine the variability from fish to flesh. For carcass containing less than 0.1 μg Hg/g the relative standard variation varied from 12 to 15%, while for carcasses containing greater than 0.1 μg Hg/g the relative standard deviation was 7–9%. In a like manner the relative standard deviations for liver and kidney were determined to be 20 and 100%, respectively. Each of these relative standard deviations is greater than that associated with the analytical procedure itself (Armstrong and Uthe 1971). In all tests of statistical significance, therefore, the larger standard deviations representing interfish variance were used. Due to the large variations in kidney levels of mercury from fish to fish, no statistical analyses were carried out on kidney values. In all tests of significance only the total mercury levels were used. The total mercury content of each sample was determined by flameless atomic absorption spectroscopy (Armstrong and Uthe 1971). Methylmercury content was determined for certain carcass samples by the method of Uthe et al. (1972). Recoveries of mercury, added either as methyl mercuric chloride or mercuric chloride, were 96 ± 2% and 96 ± 3%, respectively, for total mercury analysis, and recoveries of added methylmercury as methyl mercuric chloride were 99 ± 3% for methylmercury analysis. Attempts were made to analyze the total amount of mercury present in the water early in the experiment but were abandoned as all levels were below the detection limits of the methodology used. All values are expressed in micrograms Hg per gram on a wet weight basis. Triplicate total mercury determinations and duplicate methylmercury determinations were done.

In 1970 three experiments were carried out: in winter under the ice (January through March), in summer (June through August), and in fall (September through November). A second summer experiment was carried out in 1971. In this experiment cylindrical fiberglass cages (77-cm diameter and 77 cm long) with an open louvre at the upstream end and a 1-cm nylon seine net at the other end were used due to difficulties encountered in handling the earlier design. Although the number of fish per cage decreased with each sample, the total weight of the six fish did not change by more than 10–20% over the course of the experiment.

An attempt was made to use crayfish (*Orconectes virilis*) in a similar manner since they had been shown (Hamilton 1972; Vermeer 1972) to accumulate mercury in their abdominal muscle to levels similar to those found in white muscle of northern pike (*Esox lucius*) for the same area. Under the best of our conditions survival rate of caged crayfish was extremely poor and their use was abandoned.

Description of the River and Location of Cages

The South Saskatchewan River is a relatively slow moving (5 km/hr), entrenched river characterized by little depth (1–3 m) and shifting sandbars, especially upstream of the city of Saskatoon. Downstream of the city sandbars are less numerous and hard gravel substrata is more common. In this area rooted plants are also common. This area received sewage from Saskatoon with only shredding and minor chemical treatment (Reed 1962) and evidence of sewage was still visible at the Hague Ferry site.

The various cage sites in the river used during the course of these experiments are shown in Fig. 1. As the river is shallow, has numerous sandbars, and is subject to marked flow variations over short periods due to dam control, sites were somewhat limited and were chosen in accessible locations where severe silting was not a problem. Cages were held just below the surface of the water or just under the ice. Sites were located on the same side of the river as the outfalls except in the case of the Sutherland and Clarkboro Ferry (east side) sites. During the fall (1970) experiment, one cage (coded as IPCO) was located between the city sewer outfall and the outfall from the chemical complex. Although not in the mainstream of the sewage discharge, it was readily apparent that this cage was exposed to sewage and should be exposed to mercury from this source. Attempts to hold trout a short distance downstream of the chemical complex met with no success due to severe mortality. Cages at Diefenbaker and Pike lake sites were located above the outfalls, while cages at Sutherland, Clarkboro Ferry, Hague Ferry, and Tobin Lake sites were located below the outfalls. Analysis of a few bottom grab samples from about 100-m downstream of the chemical outfall showed varying levels of mercury (up to about 50 μg/g) while grab samples from the Clarkboro site had much less (1 μg/g) showing the presence of mercury laden deposits just downstream of the outfalls. No detailed sediment study was carried out.

Results and Discussion

In the winter experiment (Table 1) carcass mercury levels were never significantly higher than those present in the carcasses of fish taken prior to stocking. Liver levels were significantly higher only in the Clarkboro Ferry sample taken 4 weeks after initiation. Kidney levels from the same sample are suggestive of uptake occurring during the first month. All levels during the second month were not significantly different from those of fish prior to stocking. The Clarkboro Ferry liver sample (4 week) was significantly higher than corresponding levels from samples taken above the outfall sites. Mobilization of mercury from the bottom mud was still occurring during the winter, but the rate was so low that fish readily excreted all mercury taken in. It is also possible that cold depression of metabolism within the fish lowered uptake more than excretion.

During the summer (1970) experiment (Table 2) mercury uptake was apparent at all sites (levels of carcass and liver significantly higher than values found prior to stocking). Fish held at the Clarkboro and Hague ferry sites were significantly more contaminated with mercury than fish held

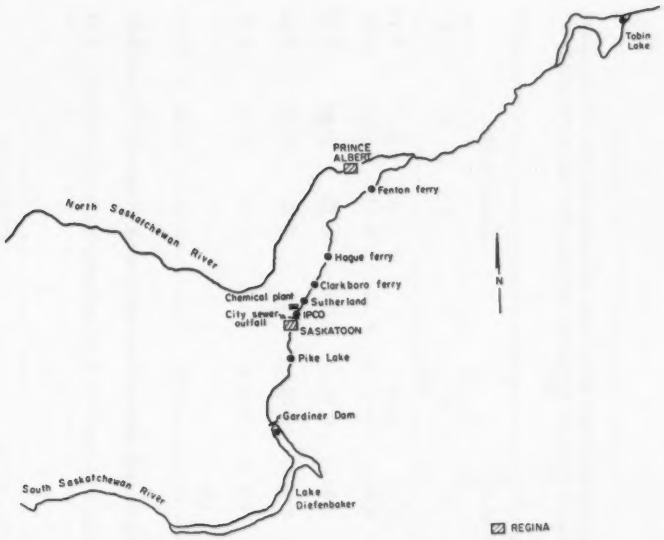

FIG. 1. Location of cage sites ● along the South Saskatchewan River system.

upstream of the outfalls, indicating a large input from the sediment beds. A few new fish were placed in the cages at the Clarkboro and Hague ferry sites at the end of July and held over August. Although the August water temperature was not significantly different from that during July and significant mercury accumulation occurred (carcass, 0.27 and 0.29; kidney, 0.61 and 0.61; for clarkboro and Hague ferry sites, respectively), the levels of mercury did not approach those found in the carcass and kidney of the fish held during July (Table 2). This suggests the occurrence of a short period of rapid mobilization of mercury. During spring the flow rates in the river were greatly increased. There was much less flow during July and August. It is possible that, during the spring runoff, the water scours the sediment and distributes a fresh layer of mercury-laden sediments dowstream of the outfall deposits. The warm temperatures of July, coupled with the extreme nutrient conditions of the river in this location and increased activity of micro- and macro-organisms, may give rise to rapid mobilization of this mercury, and then the system enters a period of lower mobilization until the next cycle. Increased activity of the fish themselves during the warm period may also enhance accumulation of mercury within the fish. The fish located at the Sutherland site, although only a short distance (about 5 km) downstream of the outfalls, did not show mercury levels significantly different from those found in fish held upstream of the outfalls. This is probably due to the fact that the location of the cage was on the side of the river opposite that of the outfalls. Laminar-type flow, preventing mixing of contaminated waters, with that on the river opposite side of the river from above the outfalls would account for the observed findings.

The accumulation of mercury was less during the fall experiment (Table 3) than the summer experiment. Again, significantly greater uptake of mercury was found in carcass and liver of fish from the Clarkboro and Hague ferry sites than was found in the fish held at Pike and Diefenbaker lakes. The cooler water temperatures of the fall experiment, coupled with decreased mobilization of mercury from the river bottom, can account for the decreased accumulation. The cage at the IPCO site (located between the city sewer outfall and the chemical plant outfall) yielded fish with with mercury levels significantly different from those at Clarkboro and Hague ferry sites but not significantly different from those at Pike Lake. This suggests that either the mercury added through sewage is of little importance in the contamination of fish downstream of the outfalls or the mercury is not present in a form for uptake by trout until it has spent some time in the river.

To explore how long the mercury in the river bottom would continue to contaminate fish the

TABLE 1. Total mercury levels (micrograms Hg per gram wet weight) of rainbow trout (*Salmo gairdneri*) held in the South Saskatchewan River during winter 1970. Each value is the mean of triplicate analyses on pooled homogenates of six fish. Statistical testing of differences between sites was carried out as described in text. No surface water temperatures were taken due to complete ice cover.

Weeks in river (stocked Jan. 15)	Carcass 0.07[a] (0.11)[b]				Liver 0.09 (0.18)[b]				Kidney 0.07r			
	2	4	6	8	2	4	6	8	2	4	6	8
Diefenbaker Lake	–[c]	0.05	0.06	0.05	–	0.08	0.05	0.08	–	0.19	0.11	0.19
Pike Lake	0.05	0.05	0.07	–	0.09	0.05	0.05	–	0.12	0.15	–	–
Clarkboro Ferry (west side)	0.06	0.06	0.06	0.07	0.15	0.21	0.07	0.07	0.28	0.32	0.19	0.23
Hague Ferry (west side)	0.05	0.05	0.06	0.05	0.08	0.12	0.06	0.08	0.22	0.22	0.16	0.17
Pike Lake vs Clarkboro Ferry (west side)	NS[d]	NS	NS	–	NS	S	NS	–				
Pike Lake vs Hague Ferry (west side)	NS	NS	NS	–	NS	NS	NS	–				

[a]Mercury content of tissue of fish (μg/g) sampled immediately prior to stocking into river.
[b]Value in brackets represents minimal value of mercury level where $P < 0.01$; that is not significantly different from level present in tissue prior to stocking.
[c]No sample.
[d]Results of Null hypothesis testing (Student's t-test with 4 df). Differences were judged significant (s) when $P < 0.01$, and insignificant (n.s.) when $P > 0.01$. In later tables a few tests yielded P's $> 0.01 < 0.05$ and were noted as such.

TABLE 2. Total mercury and methylmercury levels (micrograms Hg per gram wet weight) of rainbow trout held in the South Saskatchewan River during summer 1970. Each value is the mean of triplicate analyses on pooled homogenates of six fish. Statistical testing of differences between sites was carried out as described in text.

Weeks in river (stocked June 22)	Carcass 0.02[a] (0.06)[b]				Liver 0.08[a] (0.20)[b]				Kidney 0.20[a]			
	2	4	6	8	2	4	6	8	2	4	6	8
Surface water temperature (C)	20–21	21–23	21–22	17–18								
Diefenbaker Lake	0.05 (0.08)[c]	0.12 (0.13)	0.22 (0.11)	0.21 (0.11)	–[c]	0.15	0.35	0.32	–	0.36	0.50	0.53
Pike Lake	0.03 (0.08)	0.15 (0.07)	0.16 (0.16)	0.20 (0.12)	–	0.27	0.24	0.27	–	0.51	0.30	0.52
Sutherland (east side)	0.04 (0.04)	0.12 (0.06)	0.32 (0.16)	0.14 (0.11)	–	0.20	0.28	0.25	–	0.34	0.62	0.43
Clarkboro Ferry (west side)	0.18 (0.20)	0.35 (0.25)	0.57 (0.54)	0.56 (0.44)	–	0.57	1.11	1.26	–	0.94	1.37	1.16
Hague Ferry (west side)	0.20 (0.20)	0.26 (0.24)	0.49 (0.39)	0.37 (0.33)	–	0.65	0.87	0.97	–	0.70	1.02	0.77
Tobin Lake	– (–)	0.06 (0.04)	0.13 (0.10)	0.18 (0.08)	–	0.14	0.23	0.28	–	0.17	0.22	0.34
Pike Lake vs Sutherland	NS[d]	NS	S[d]	NS	–	NS	NS	NS				
Pike Lake vs Clarkboro Ferry (west side)	S	S	S	S	–	NS[f]	S	S				
Pike Lake vs Hague Ferry (west side)	S	S	S	S	–	S	S	S				
Pike Lake vs Tobin Lake	–	NS	NS	NS	–	NS	NS	NS				
Diefenbaker Lake vs Tobin Lake	–	NS	S	NS	–	NS	NS	NS				

[a]See Table 1.
[b]See Table 1.
[c]See Table 1.
[d]See Table 1.
[e]Methylmercury content of tissue. Value in brackets represents the mean value of duplicate analyses.
[f]$0.05 > P > 0.01$.

TABLE 3. Total mercury levels (micrograms Hg per gram wet weight) of rainbow trout held in the South Saskatchewan River during fall 1970. Each value is the mean of triplicate analyses on pooled homogenates of six fish. Statistical testing of differences between sites was carried out as described in the text.

Week in river (stocked Sept. 10)	Carcass 0.06[a] (0.10)[b]				Liver 0.11[a] (0.27)[b]				Kidney 0.19[a]			
	2	4	6	8	2	4	6	8	2	4	6	8
Surface water temperature (C)	13–14	13–14	4–6[c]	3–6[c]								
Diefenbaker Lake	0.05	0.10	0.15	0.11	0.07	0.12	0.19	0.14	0.11	0.18	0.23	0.14
Pike Lake	0.07	0.14	0.13	0.10	0.15	0.19	0.18	0.20	0.12	0.39	0.32	0.19
IPCO	0.10	0.14	0.14	0.14	0.19	0.32	0.28	0.26	0.24	0.39	0.33	0.39
Clarkboro Ferry (west side)	0.17	0.21	0.25	0.22	0.33	0.51	0.48	0.40	0.27	0.46	0.60	0.37
Hague Ferry (west side)	0.12	0.26	0.29	0.26	0.37	0.50	0.48	0.40	0.28	0.58	0.56	0.34
Tobin Lake	0.16	0.24	0.15	0.12	0.18	0.34	0.22	0.23	0.25	0.41	0.27	0.20
Pike Lake vs IPCO	NS[d]	NS	NS	NS	NS	NS	NS	NS				
Pike Lake vs Clarkboro Ferry (west side)	S[d]	S	S	S	NS[e]	S	S	S				
Pike Lake vs Hague Ferry (west side)	NS	S	S	S	S	S	S	NS				
IPCO vs Claboro	S	S	S	S	NS	NS	NS	NS				
Diefenbaker Lake vs Tobin Lake	S	S	NS	NS	S	S	NS	NS				

[a]See Table 1.
[b]See Table 1.
[c]Broken ice along river shorelines.
[d]See Table 1.
[e]$0.05 > P > 0.01$.

summer experiment was repeated on a smaller scale in 1971. Also, another cage (Clarkboro east) was placed at the Clarkboro Ferry site on the side of the river opposite the outfalls to check for occurrence of a laminar-type flow in the river as suggested by the findings (mentioned above) of low mercury levels in fish held a short distance downstream of the outfalls on the side of the river opposite the outfalls (Table 3, Sutherland site). The results of this experiment (Table 4) showed that the level of uptake found downstream of the outfall (Clarkboro Ferry west) was not significantly different from levels obtained the previous year. The Pike Lake site did not show any significant difference between years. Levels at both sites in 1971 were somewhat less than in 1970, but probably represents overall changes in conditions in the river. However, as stated above, differences were not significant at the 1% level. The cage located at Clarkboro Ferry east site yielded fish with mercury levels significantly lower than those held at Clarkboro Ferry west site. This definitely shows the existence of a laminar-type flow within the river and shows that this lack of mixing of mercury-laden waters extends at least as far as Clarkboro (approximately 15 km). This explains why the Sutherland site (Table 3), although located downstream of the outfalls, did not yield contaminated fish.

During the summer and fall experiments fish were held in Tobin Lake (the first lake downstream of the outfalls) with fish held in Diefenbaker Lake (the first lake upstream) as a control. No large increase in levels of mercury were found in caged trout from Tobin as compared to Diefenbaker Lake (Tobin was significantly higher in 3 of 7 cases). This suggests that mercury deposits from the outfalls are not contributing to mercury levels in Tobin Lake to a significant degree.

Westoo (1966) found that the bulk of mercury in fish is present as the monomethylated derivative. Analyses of fish carcass from the summer experiment (Table 2 and 4) showed that this was also true of the caged fish. Analyses of rainbow trout exposed to inorganic mercury, phenylmercury acetate, or

TABLE 4. Total and methylmercury (micrograms Hg per gram wet weight) of rainbow trout held in South Saskatchewan River during summer 1971. Each value is the mean of triplicate analyses on pooled homogenates of six fish. Statistical testing of differences between sites was carried out as in the text.

Weeks in river (stocked June 15)	Carcass 0.06[a] (0.10)[b]			Liver 0.11[a] (0.27)[b]			Kidney[a] 0.19		
	2	4	6	2	4	6	2	4	6
Surface water temperature (C)	18–19	20–21	21–23						
Pike Lake	0.08 (0.06)	0.09 (0.07)[c]	–[c]	0.06	0.08	–	0.16	0.18	–
Clarkboro Ferry (east side)	0.07 (0.06)	0.09 (0.07)	0.10 (0.09)	0.10	0.12	0.17	0.18	0.24	0.19
(west side)	0.16 (0.15)	0.22 (0.20)	0.44 (0.39)	0.30	0.34	0.85	0.30	0.34	1.01
Pike Lake vs Clarkboro Ferry (east side)	NS[d]	NS	–	NS	NS	–			
Pike Lake vs Clarkboro Ferry (west side)	NS	S[d]	–	S	S	–			
Pike Lake (1970) vs Pike Lake (1971)	NS	NS	–	NS	NS	–			
Clarkboro Ferry (west side, 1970) vs (west side, 1971)	NS	NS	NS	–	NS[f]	NS			

[a]See Table 1.
[b]See Table 1.
[c]See Table 1.
[d]See Table 1.
[e]Methylmercury content of tissue.
[f]$0.05 > P > 0.01$.

methyl mercuric chloride showed that only those fish exposed to methyl mercuric chloride accumulated methylmercury in their tissues even though all fish showed accumulation of mercury (Uthe et al. 1972), suggesting that the caged trout accumulate methylmercury from their surroundings and do not methylate mercury after intake.

In conclusion, it can be stated that caged fish are good monitors of mercury pollution. The levels attained were not as high as those found in native fish, but highly significant differences were found above and below the major source of contamination. These studies have shown that the cessation of mercury discharge into the river does not lead to elimination of mercury uptake in fish. The microbial mobilization of mercury can be expected to continue as long as mercury is present in the biologically active portion of the river bottom. The evidence presented indicates that the river has limited ability to remove significant portions of deposits in a short time and, as in the Swedish situation (Jernelov 1969), it may be years before these man-made deposits are eliminated. The minimal differences found in fish from cages from Tobin and Diefenbaker lake sites is suggestive, however, that the sediments from the Saskatoon area did not contribute to the mercury taken up by native fish in Tobin Lake. The presence of fish with contamination levels of mercury (>0.5 μg/g in many lakes (Tam and Armstrong 1972) where no man-made mercury input is apparent is well documented, and it may be that contaminated fish in the lower reaches of the Saskatchewan River system now result mainly from other factors.

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Behavior of Young Atlantic Salmon (*Salmo salar*) Exposed to or Force-Fed Fenitrothion, an Organophosphate Insecticide

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SYMONS, P. E. K. 1973. Behavior of young Atlantic salmon (*Salmo salar*) exposed to or force-fed fenitrothion, an organophosphate insecticide. J. Fish. Res. Board Can. 30: 651–655.

Exposure of young Atlantic salmon (*Salmo salar*) to 1.0 ppm fenitrothion for 15–16 hr caused a 50% decrease in the number holding territories 6 days following treatment. Some severely affected fish also swam stiffly and ceased feeding, but these effects disappeared within 48 hr following return to clean water. Territories were not reclaimed for approximately 2–3 weeks. Exposure to 0.1 ppm fenitrothion for 15–16 hr caused a lesser (20%) reduction in numbers of fish holding territories.

When mealworms (*Tenebrio* sp.) injected with 2–5-μliters pure (100%) fenitrothion were force-fed to young salmon, 50% were regurgitated 8–12 hr afterwards. Almost all mealworms containing 10–20-μliter fenitrothion were regurgitated. The proportion of worms regurgitated remained constant during a week of daily force-feedings but, 24 hr after the third or fourth feeding, all fish except controls could be made to flex tetanically by rapping on the aquarium, and they made little attempt to escape a dipnet.

SYMONS, P. E. K. 1973. Behavior of young Atlantic salmon (*Salmo salar*) exposed to or force-fed fenitrothion, an organophosphate insecticide. J. Fish. Res. Board Can. 30: 651-655.

L'exposition de jeunes saumons atlantiques (*Salmo salar*) à une teneur de fénitrothion de 1.0 ppm durant 15–16 h cause une diminution de 50% des individus qui maintiennent un territoire, 6 jours après le traitement. De plus, certains poissons plus sérieusement affectés nagent avec raideur et cessent de se nourrir, mais ces symptômes disparaissent en dedans de 48 h après retour en eau propre. Ce n'est que 2 ou 3 semaines après, que les territoires sont occupés de nouveau. Une exposition de 15–16 h à une teneur de fénitrothion de 0.1 ppm cause une diminution moins forte (20%) des poissons qui occupent un territoire.

Lorsqu'on gave des saumonneaux de vers de farine (*Tenebrio* sp.) injectés de 2–5-μliters de fénitrothion pur (100%), ils en régurgitent 50%, 8–12 h après. Ils régurgitent presque tous les vers contenant 10–20 μliter de fénitrothion. La proportion des vers régurgités demeure constante durant une semaine de gavages quotidiens, mais 24 h après la 3ième ou 4ième opération, un coup sec sur la paroi de l'aquarium provoque des flexions tétaniques chez tous les poissons, sauf les témoins, et ils ne font aucun effort pour éviter une épuisette.

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FOREST spraying of the organophosphate insecticide fenitrothion (also known as Sumithion®) at 140–210 g/ha (2–3 oz/acre) is reported to cause little direct mortality of young Atlantic salmon, *Salmo salar* (Hatfield and Riche 1970). However, the insecticide may alter behavior of the fish. Twenty-four-hour exposure of salmonids to 1.0 ppm fenitrothion suppresses aggressive and territorial behavior (Bull 1971), increases susceptibility to predation (Hatfield and Anderson 1972), and inhibits learning (Hatfield and Johansen 1972). The present report describes effects of brief (15–16-hr) exposure to fenitrothion on territorial behavior and locomotion of juvenile Atlantic salmon (parr), and also some effects on bodily functions of fish that have ingested the insecticide.

Materials and Methods

Either a stock emulsion of 2.77-g fenitrothion/liter of water or a colloidal solution of 4-g fenitrothion/liter of Corexit® 7664 in distilled water (1:50 weight/weight ratio fenitrothion:Corexit® 7664) was used to prepare 20-liter treatment baths at concentrations of 1.0 ppm and 0.1 ppm fenitrothion. The 24-hr LC50 concentration of fenitrothion in Corexit® 7664 for Atlantic salmon fry is 4.2 ppm, and the incipient lethal level (ILL) is 2.0 ± 0.25 (Wildish et al. 1971). Corexit® 7664 causes no mortality of young salmon in 96 hr at concentrations

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of 500 ppm (Zitko and Carson 1969). Water was soft (13–14-mg $CaCO_3$/liter) with *p*H 7.0–7.1.

Two artificial streams with windows were used to observe behavior of the fish. These streams had areas of 5.5 m^2 and 31 m^2, mean widths of 60 cm and 100 cm, respectively, and current velocities of 20–80 cm/sec, depending upon location. The bottom of the smaller stream was covered with gravel, rocks, and small boulders, but the larger contained only small boulders or cement construction blocks for shelter.

Young Atlantic salmon (8.1–14.4 cm, averaging ~12 g) were taken either from a holding tank or from one of the two streams in which they had resided at least 5 days. One of each pair of fish, chosen randomly, was placed in a treatment bath, and the other in a control bath with water alone. These baths were lined with plastic and contained an airstone. They were filled with water from the smaller stream at 1645 hr, immediately before beginning treatment or handling of the fish. The fish were kept in the baths overnight for 15–16 hr only because, if absence had continued through the day, fish remaining in the streams might have usurped the vacated stations or territories of the absentees. Only one pair of fish was treated and handled per night.

A total of 69 fish were used, of which 10 were reused (one was reused twice), usually as a control first. None was exposed to fenitrothion more than once, and, for the three fish treated first and then reused as controls, the shortest time between treatment and reuse was 55 days. This provided four lots of fish of 10 pairs each in a two-by-two design, with two treatment concentrations of 0.1 ppm and 1.0 ppm fenitrothion and two origins: holding tanks or artificial streams. No fish died during treatment, though results from one control fish were discarded because of an irregularity in handling, and another control fish died through accident one day following treatment.

Distance and direction moved by each fish were observed for 10 min immediately after introduction or return to streams at 0830, and thereafter usually twice daily for 7 days. In a few cases, observations were continued for 20–30 days. During observations the status of the fish as a territorial dominant or subdominant, or as a nonterritorial fish with a home station, or as a transient without territory or home, was noted.

Most tests were made in summer when water temperatures ranged from 13.5 to 22.0 C, but seven pairs of fish were tested in the autumn at 7–16 C, or in winter at constant 2 C. Photoperiods were 15–16-hr light in summer, light commencing at 0430 AST, and 8½ hr in winter with light commencing at 0830 AST. The population in the larger stream was maintained at eight, while in the smaller stream it ranged from five to 15. The fish were fed mealworms (*Tenebrio* sp.) and marine amphipods (*Gammarus oceanicus*).

The effects of ingesting the insecticide were tested in separate experiments in which the fish were anesthetized in M.S. 222 and force-fed mealworms containing 100% fenitrothion. Three experiments were performed to investigate effects of ingesting different quantities of fenitrothion in single or repeated meals. In the first experiment, each of 20 fish was fed a single mealworm containing 0 (control), 2, 5, 10, or 20 µliters fenitrothion. In the second, each of 10 fish was fed a single mealworm containing 0, 2, 3, 5, or 20 µliters fenitrothion daily for 7 or 8 days. In the third, each of two fish received five mealworms daily for 4 days, each worm containing 1 µliter fenitrothion.

Force-feeding was necessary because young salmon would not voluntarily eat mealworms injected with 100% fenitrothion. Forceps were used to insert mealworms beyond the throats of anesthetized fish. If a mealworm was not retained for 1 hr following recovery of the fish from the anesthetic, the feeding was repeated. Mealworms not regurgitated within 1 hr were always retained at least 6 hr.

Fenitrothion was injected into mealworms with a 10-µliter syringe, the needle being inserted through the posterior end to the head where either the full dose was discharged or, in the last two experiments, the syringe was discharged as the needle was withdrawn, distributing the insecticide through the worm. There was little or no leakage through the needle hole.

With the exception of the two fish in the last experiment, which were kept individually in 300-liter tanks with recirculating water, all fish used in ingestion experiments were kept individually in 17.5-liter glass aquaria with standing, aerated water. Aquaria were emptied and rinsed daily. Temperature was maintained at 16–17 C.

Results

Behavioral Effects in the Stream

There was a tendency for treated fish of all four lots to move less than their paired controls during the first 24 hr after exposure to the insecticide (Fig. 1), but this result was not sufficiently consistent to be significant ($0.05<P<0.1$; sign-test on combined data). Treated fish showed no consistent trend to move up- or down-stream more than their controls.

Since approximately 5 days are required for young Atlantic salmon to acquire a territory (Symons 1971), no differences in territorial behavior between treated and control fish newly introduced to the stream were expected or found in the 7 days following introduction.

Of those removed from the stream, control fish of both lots combined showed a slight but insignificant ($P>0.1$; chi-square test) decline in percentage defending territories during the first 24 hr, after which there were no further changes:

Control fish	Before removal Day −1	Days after removal 0	+1	+2 to +7 (mean)
% territorial	63	55	50	50

(days −1 to +1, 19 fish; days +2 to +7, 18 fish)

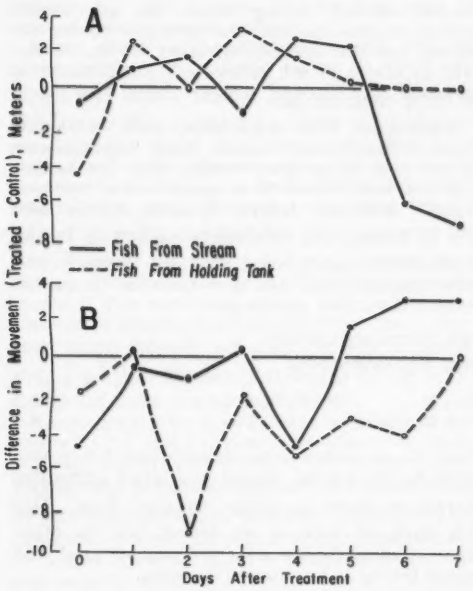

FIG. 1. Median difference in movement between treated and control (0-line) parr immediately after treatment (day 0) and on the 7 days following. A, fish treated in 0.1 ppm (15–16 hr); B, fish treated in 1.0 ppm.

Treated fish from the stream at both concentrations of fenitrothion showed a larger decline in numbers defending territories after return than did controls, and this decline continued after the first 24 hr:

Treated fish	Before treatment Day −1	Days after treatment 0	+1	+2 to +7 (mean)
0.1 ppm fenitrothion % territorial (10 fish)	90	70	60	45
1.0 ppm fenitrothion % territorial (10 fish)	70	40	20	17

This decline between day −1 and day +1 (all treated fish combined) was significant ($P<0.01$; chi-square test). Comparison of treated fish with controls was complicated because, by chance, more of the treated fish were territorial before treatment than were controls before handling. Although this situation was reversed by day +2, and the difference increased to day 6, it never became statistically significant ($0.05<P<0.1$; chi-square test, data not shown).

Effects of the two concentrations of insecticide are more easily compared if only those fish that were territorial on the day before removal from the stream, and those fish that survived to the sixth day, are considered:

Treatment	No. territorial Day −1	Day +6	% decrease
Control	11	7	36
0.1 ppm fenitrothion	9	4	55
1.0 ppm fenitrothion	7	1	85

Though these numbers are too small for meaningful statistical analysis, they are consistent with a hypothesis that increasing concentrations of fenitrothion progressively disrupt territorial behavior. Exposure to 1.0 ppm fenitrothion for 15–16 hr apparently increased the proportion of territorial fish that were expected to be seen without territories 6 days later by about 50%.

Exposure to 1.0 ppm fenitrothion affected some fish more severely than others. A few fish ceased feeding and swam stiffly, holding their fins fully distended. Swimming became normal 24 hr after exposure, but feeding was not resumed sometimes until 2 days after treatment. All control fish fed and swam normally within a few minutes of being placed in the stream.

Two of the most severely affected fish that had been territorial dominants before treatment were left in the stream for 20–30 days to observe recovery. After exposure to 1.0 ppm fenitrothion, both swam stiffly, ceased feeding, and became completely subordinate. They did not regain their territories until the 11th and 24th day after treatment (temperatures during recovery were 20 and 7 C, respectively). The paired controls for each of these fish continued to defend territories for at least 4 days after replacement in the stream.

Two of the fish treated in 0.1 ppm fenitrothion in July and August, when temperatures ranged between 16 and 22 C, were reused as controls in October and January, when temperatures had decreased to 6 and 2 C, and another fish treated at 1.0 ppm in October was reused as a control in February at 2 C. These fish appeared normal and healthy, and their behavior was indistinguishable from that of all other controls, none of which had been treated previously.

Effects of Ingesting Fenitrothion

None of the fish in any of the three ingestion experiments died, but regurgitation occurred in every experiment and was the first response observed. All regurgitations attributed to the insecticide occurred at least 1 hr and, usually, 8–12 hr after force-feeding.

Results of force-feeding single mealworms in a single meal demonstrated that worms containing between 2 and 5 µliters of 100% fenitrothion had a 50% chance of having at least a part regurgitated (Table 1). Almost all fish receiving 10–20-µliters fenitrothion regurgitated complete undigested worms, but those force-fed worms with no fenitrothion regurgitated none.

Fish force-fed single mealworms daily for 7 or 8 days did not alter their frequency of regurgitating with time. The percentage of worms regurgitated depended broadly only on the quantity of fenitrothionin the worms:

µliters fenitrothion	0	2	3	5	20
% worms regurgitated	0	40	50	40	80
Number of worms fed (2 fish at each level)	15	15	15	15	14

In addition to regurgitation, four other effects were noted in this experiment. Digestion appeared to be slowed or prevented by the fenitrothion since worms were frequently regurgitated intact and apparently untouched by digestive processes after at least 8 hr in the stomach. Feces frequently were formed of strings of white mucous. All treated fish made little attempt to avoid the dipnet and, finally, convulsive flexing developed.

Convulsive flexure appeared, usually 24 hr after the third or fourth feeding of treated worms, in response to a rap on the aquarium or immersion of the fish in the anesthetic. Severity of flexing varied from day to day and ranged from a momentary sigmoid flexure to a rigid semicircular posture held for several seconds during which the fish usually turned ventral-side up. The strongest flexure observed was held for approximately 30 sec. Flexure was as severe in fish receiving 2 µliters/worm as in those receiving 20.

Feeding of five mealworms, each containing 1-µliter fenitrothion, caused fewer regurgitations in the first 24 hr after feeding than did feeding 5-µliters fenitrothion in a single worm (compare Table 1 with data below). However, by the third day of feeding five mealworms each with 1-µliter fenitrothion, regurgitation increased. Some worms were disgorged that had been force-fed to the fish more than 24 hr previously:

No. worms regurgitated 24 hr after meal number	1	2	3	4
Fish #1	0	1.3	3	2.3
Fish #2	1	0	6.6	4.7

Fish #2, in addition to regurgitating 6.6 worms after the third meal, passed two intact undigested worms wrapped in white mucosal feces. This fish displayed little or no flexing, but the other showed some flexing after the second meal and severe flexing 24 hr after the last meal.

Table 1. Effect of single mealworms injected with varying quantities of fenitrothion on salmon parr regurgitation.

µliters fenitrothion in worm	No. fish regurgitating		
	0 worm	10–50% of worm	Whole worm
0	5	0	0
2	4	1	0
5	1	3	1
10	0	1	4
20	1	0	4

Discussion

Wildish et al. (1971) estimated that a stream 30 cm deep should have a concentration of 0.045 ppm of fenitrothion immediately after spraying at 140 g/ha (2 oz/acre). The experiments reported here indicate that concentrations would have to be approximately double this for at least 15–16 hr before the insecticide caused 20% of the salmon parr to desert their territories. Little is known of the levels reached and maintained in the days following forest spraying.

Disruption of territorial and other behavior might be augmented by the ingestion of insects killed by fenitrothion. Hatfield and Riche (1970) found concentrations of fenitrothion as high as 2.23 ppm in the stomachs of parr caught in a sprayed brook. In well-fed 12-cm parr the volume of the stomach and contents is approximately 0.5 ml (personal observation) and, therefore, a concentration of 2.23 ppm represents a quantity of approximately 0.001-µliter fenitrothion in the stomach. Force-feeding 2000 to 20,000 times this quantity failed to kill parr. Since parr severely affected by the insecticide in the water ceased feeding, they would not accumulate fenitrothion by ingestion. Therefore, while ingestion of contaminated insects might enhance disruption of the territorial behavior of fish weakly affected by fenitrothion in the water, death from combined

exposure to and ingestion of the insecticide in sprayed streams seems unlikely.

Severe effects of ingesting daily meals of 2–20-μliters fenitrothion included failure to avoid the dipnet and tetanic flexing. Kerswill and Edwards (1967) were able to pick up a brook trout (*Salvelinus fontinalis*) found in a stream that had been sprayed with DDT. They also reported that salmon and trout in streams sprayed with DDT may become beached as a result of extreme convulsive activity just before death, and Saunders (1969) reported the same behavior for salmon and trout exposed to a mixture of the fungicide "nabam" and the insecticide "endrin." This convulsive activity differed from the behavior of salmon parr force-fed fenitrothion; they never swam excitedly but always moved sluggishly and, if startled, became immobilized for up to 30 sec by the tetanic semicircular flexure.

Recovery of salmon parr from the effects of exposure to 0.1 and 1.0 ppm fenitrothion for 15–16 hr would appear to be complete in 3 weeks or less. Feeding and swimming behaviors, if affected at all by such exposure, return to normal within 24–48 hr. Desertion of territories after exposure may continue for 6 days following return to clean conditions, but even severely affected fish exposed to 1.0 ppm fenitrothion reclaimed territories 2–3 weeks after exposure.

Long-term or delayed effects have been observed after spraying of streams with DDT in spring. Mortalities of salmon parr increased abnormally in the fall as temperatures fell (Kerswill and Edwards 1967). Such effects were not observed among three parr exposed to fenitrothion in the summer or fall and reused as controls at lower temperatures 2–4 months later. Though this absence of effect needs verification, the present experiments indicate that no behavioral effects of 15–16 hr exposure to 1.0 ppm fenitrothion are to be expected after 3 weeks.

Although, from the results of the experiments, spraying streams with fenitrothion at 140 g/ha (2 oz/acre) would appear unlikely to have much influence on behavior and immediate survival of salmon parr, production of salmon in sprayed streams might still be adversely affected. Spraying of streams with insecticides is often followed by a decrease in biomass of aquatic insects (Elson et al. 1972). Spraying streams with DDT may sometimes reduce the volume of food consumed by large parr (> 9 cm) for 1–2 months afterwards (Keenleyside 1967). In a laboratory stream reduction of food supply results in emigration of smaller subordinate parr from the area and increases the chances that territorially dominant parr will desert their territories (Symons 1971). Salmon parr emigrating from a small natural stream in the fall after spraying of fenitrothion in the spring apparently survive poorly because, while 1.3–2.8% of spring migrating smolts are recaptured as adults (J. W. Saunders personal communication), only two of 1411 parr migrating in the fall have ever been so reported.

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Sensitivity of American Eels (*Anguilla rostrata*) and Atlantic Salmon (*Salmo salar*) to Weak Electric and Magnetic Fields

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ROMMEL, S. A. JR. AND J. D. MCCLEAVE. 1973. Sensitivity of American eels (*Anguilla rostrata*) and Atlantic salmon (*Salmo salar*) to weak electric and magnetic fields. J. Fish. Res. Board Can. 30. 657–663.

Two long distance migrating fishes, the American eel (*Anguilla rostrata*) and the Atlantic salmon (*Salmo salar*), consistently showed conditioned cardiac responses to weak electric fields applied perpendicular but not parallel to their bodies. In fields with a power density of 0.11×10^{-3} pW/cm^3, 63% of the eels tested were successfully conditioned, as were 56% of the salmon tested at 0.9×10^{-4} pW/cm^3. At greater field intensities greater percentages of salmon and eels were successfully conditioned. However, at two lower intensities fewer salmon and no eels responded. These fields are of the magnitude that occur naturally in ocean currents. As geoelectric fields are perpendicular to the water motion that generates them, this sensitivity might allow a migrating fish to align itself upstream or downstream in an ocean current in the absence of fixed references. Results of magnetic conditioning experiments in eels were equivocal. Attempts to condition salmon to weak magnetic fields were unsuccessful.

ROMMEL, S. A. JR. AND J. D. MCCLEAVE. 1973. Sensitivity of American eels (*Anguilla rostrata*) and Atlantic salmon (*Salmo salar*) to weak electric and magnetic fields. J. Fish. Res. Board Can. 30. 657–663.

Lorsque des poissons de deux espèces à migrations étendues, l'anguille d'Amérique (*Anguilla rostrata*) et le saumon atlantique (*Salmo salar*), sont exposés à de faibles champs électriques appliqués perpendiculairement, mais non parallèlement, au corps, il s'ensuit toujours une réponse cardiaque conditionnée. Dans des champs de densité de pouvoir de 0.11×10^{-3} *p*W/cm^3, le conditionnement se produit chez 63% des anguilles. Il en est de même dans le cas de 56% des saumons exposés à un champ de 0.9×10^{-4} *p*W/cm^3. A de plus hautes intensités, la proportion des saumons et des anguilles conditionnés avec succès est plus grande. Par ailleurs, moins de saumons et aucune anguille ne réagissent à deux intensités plus faibles. Ces champs sont de l'ordre de grandeur de ceux qui existent naturellement dans les courants océaniques. Puisque ces champs géoélectriques sont perpendiculaires à la direction des mouvements de l'eau qui les produisent, il est possible que cette sensibilité permettre à un poisson migrateur de s'orienter, dans un sens ou dans l'autre, dans un courant marin, en l'absence de tout point de repère. Des essais de conditionnement de l'anguille dans un champ magnétique ont donnés des résultats contradictoires. Des essais semblables sur le saumon ont été sans succès.

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THE open ocean guidance mechanisms for long distance migrations of fishes remain puzzling despite extensive work which has shown that vision and olfaction play an important part in the lake and river movements of some species (Hasler 1966, 1971). However significant olfactory and visual stimuli may be, they alone cannot account for the open water movements of some fishes (Royce et al. 1966; Tesch 1967; Deelder 1952). Harden Jones (1968) emphasized the need to investigate the hypothesis that water currents are important in orientation during fish migration. Royce et al. (1968) reported that Pacific salmon (*Oncorhynchus* sp.) migrate along ocean currents, but swim faster than the current speed, and Marty (1959) reported the same behavior in Atlantic herring (*Clupea harengus*). The electric field generated by waters moving through the geomagnetic field was strongly suggested as a means by which

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salmon might maintain their orientation to the waters currents (Royce et al. 1968). Deelder (1952) stated that geoelectric information from ocean currents may orient migrating European elvers (*Anguilla anguilla*). The geoelectric field is directional and has polarity, with the major portion of the field perpendicular to the direction of water motion. This directional information could guide these fishes along the observed pathways; however, the electro-sensitivity of the Pacific salmon, the European eel, and the herring have not been investigated.

Although the long distance migratory routes of American eels (*Anguilla rostrata*) and Atlantic salmon (*Salmo salar*) are essentially unknown, it seems likely that portions of these routes are along or parallel to major ocean currents. Therefore if the sensitivity of these fishes were sufficient, they could use geoelectric fields to determine water current direction and hence maintain their orientation to it. One objective of this research was to determine if the American eel and Atlantic salmon are sensitive to electric fields of naturally-occurring intensities.

Fish might orient by the potentials generated in their bodies as they move through the geomagnetic field (Murray 1962; Thornton 1932). Tesch (1967) mentioned magnetic compass orientation as a possible navigational aid for European eels. Recently Gleiser and Khodorkovsky (1971) have suggested that the European elver is sentitive to the earth's magnetic fields. A second objective of this research was to determine if Altantic salmon and American eels are sensitive to d-c magnetic fields of geomagnetic strength.

Methods

Searun Atlantic salmon parr (15–24 cm body length) were obtained from the Craig Brook National Fish Hatchery, Orland, Maine, and were held at 10 ± 1 C, at which temperature all salmon experiments were conducted. American eels (35–56 cm body length), trapped in fresh and estuarine waters of Maine and New Brunswick, were supplied by a commercial dealer. All eels were held and experiments were conducted at 20 ± 1 C. The resistivity of the well water used in fresh-water experiments was 4000 ohm-cm. For experiments in salt water, well water was made saline by the addition of synthetic sea salts (Instant Ocean). The two salinities selected correspond to one and two orders of magnitude reduction in resistivity of the well water, i.e., 400 ohm-cm and 40 ohm-cm (approximately 1‰ and 17‰, respectively). Eels and salmon tested in 400 ohm-cm water were transferred directly from fresh water and held for at least 2 days prior to testing. Eels tested in 40 ohm-cm water were first held in 400 ohm-cm water for 2 days and then transferred to 40 ohm-cm water and held for at least 5 days prior to testing. Salmon tested in 40 ohm-cm water were acclimated by increasing the salinity approximately 1‰ per day. They were held in 40 ohm-cm water at least 5 days prior to testing.

To determine a response to the electric or magnetic field, a cardiac deceleration was attempted by classical conditioning in response to presentation of either field (McCleave et al. 1971; Rommel and McCleave 1972).

Cardiac electrodes were implanted as previously described (McCleave et al. 1971; Rommel and McCleave 1972). Immediately after implantation of electrodes each fish was placed in a plexiglass aquarium 100 × 30 × 37 cm. To maintain the long axis of the fish parallel to the long axis of the tank, each fish was restricted by a plastic mesh cage which allowed the fish just to turn around and move back and forth. After a few minutes each fish maintained a position in the center of the cage but resting on the bottom. Electrocardiograms were recorded on a Physiograph Model 4-A or a Grass Model 7 Polygraph.

The electric field was applied by injecting a known electric current density into the tank via two stainless steel plates situated at the ends or sides of the tank. Measurements of the field showed less than ± 1% non-uniformity. Each presentation of the electric field was monitored by a microammeter in series with the tank. Distortion in the electric field by the mesh of the plastic cage was less than ± 1%, more than 2 mm from the plastic.

The magnetic field was produced by one of three sets of mutually perpendicular Helmholtz coils, each 1 m in diameter. Measurements of the field with a Hall effect gauss meter showed a field uniformity of better than ± 3%. Electric current for the coils was supplied by a filtered dc power supply (<1% ripple). Each presentation of the field was monitored by a calibrated ammeter in series with the coils. There was no distortion of the magnetic field by the mesh of the plastic cage.

Eighty training trials were attempted on each fish. A trial consisted of the presentation of either the electric or magnetic field (conditioned stimulus, CS) for three consecutive heartbeats, then presentation of a 1–2 vac shock (unconditioned stimulus, UCS) after which both were turned off. Fifteen trials per set were arbitrarily chosen in the earliest eel experiments, but these data showed a diminished response before the end of each set. Thereafter trials were presented in sets of eight for salmon and ten for eels. Sets were approximately 50 min apart. An irregular interval of 40–70 sec between trials was used to avoid temporal conditioning. Some fish pulled out their electrodes, the electrodes shorted out, or the EKG was unreadable due to noise before 80 trials were realized. A minimum of 50 trials for eels and 48 for salmon was selected as the smallest acceptable sample size for the statistical analysis.

The data from each fish were statistically treated separately. As an index of heartbeat rate, the interbeat interval was measured to the nearest 0.04 sec. The first two beats after the CS was turned on were designated the first and second test beats, and the beat immediately prior to the field was designated the test reference beat (Fig. 1). The change in rate from the reference beats to

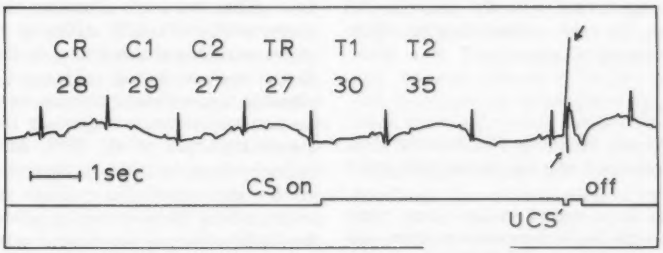

FIG. 1. EKG of an American eel during one training trial showing apparent conditioned cardiac deceleration, presentation of weak electric field (CS) (0.167 $\times$ 10^{-2} μA/cm^2), unconditioned stimulus (UCS), and designation of test and control heartbeats. CR = control reference beat; C1, C2 = control beats; TR = test reference beat; T1, T2 = test beats. Numbers are interbeat intervals (in millimeters at 25 mm/sec chart speed from original EKG record). Arrows mark artifacts caused by the UCS.

each test beat was calculated as a percentage. Since the mean percent change of any two beats compared to a prior reference beat might not be zero, the basis for statistical comparison was a series of two control beats and a control reference beat just prior to them.

The mean percent change in test beat one was compared with the mean percent change in control beat one, and similarly test beat two with control beat two. A one-tailed, Wilcoxon's signed ranks test at the 95% level was used to test the null hypothesis that no difference between the control and test means existed. The alternate hypothesis was that the test means were greater than the control means, if conditioning occurred.

One hundred twenty seven salmon and eels were tested either parallel or perpendicular to an electric field in three water resistivities and five electric current densities. Fewer tests were conducted with the electric field parallel to the fish's body, since in none of these cases was there any indication of learning.

The magnetic field experiments were the same for both species. Twelve salmon were tested, four at each of three field conditions: cancellation of the vertical magnetic field plus 0.5 gauss in the opposite direction; cancellation of the north–south magnetic field plus 0.5 gauss in the opposite direction; 0.5 gauss field in the east–west direction. Twenty-two eels were tested in the same field conditions: eight vertical; eight north–south; six east–west. The local horizontal magnetic field was 0.17 gauss and the local vertical component 0.38 gauss. Each fish was oriented by the mesh cage in the magnetic north–south direction. All magnetic field experiments were conducted in 4000 ohm-cm water.

Results

Electric Fields

Twenty-five of 55 eels tested with the electric field perpendicular to the long axis of their bodies exhibited significant cardiac conditioning (Table 1). The numbers of fish that exhibited significant conditioning (learners) decreased with decreasing electric current density and decreasing electric field intensity. Twenty of 23 eels tested in fields with a power per unit volume[2] (hereafter referred to as power density) at least 0.11 $\times$ 10^{-2} pW/cm^3 were successfully conditioned (examples in Table 2). Fewer animals responded in fields of 0.11 $\times$ 10^{-3} pW cm^3, and no eels responded in fields of 0.11 $\times$ 10^{-4} pW/cm^3 and lower.

Eight eels were tested with the electric field parallel to the long axis of their body. In none of these fish was cardiac conditioning successful (Table 1). The fact that learning was not exhibited in the parallel fields and lowest intensity perpendicular fields showed that extraneous factors associated with the experimental technique were not responsible for the observed conditioning.

Twenty-nine of 55 salmon tested with the electric field perpendicular to the body axis exhibited significant conditioning (Table 1). The same general trend of decreasing numbers of learners with decreasing power density (from decreasing field intensity or current density) was evident for salmon as for eels. However, the salmon threshold was lower and not as distinct. Only 11 of 19 salmon tested at power densities of 0.9 $\times$ 10^{-2} and 0.9 $\times$ 10^{-3} pW/cm^3 were learners (Table 1,3), and 10 of 18 at 0.9 $\times$ 10^{-4} pW/cm^3 learned. At the two lowest power densities seven of 18 salmon were conditioned, yet no eels were conditioned at the comparable power densities.

[2]Power density equals electric current density times total electric field intensity. In a resistor where velocity with respect to the magnetic field is zero the total electric field intensity equals electric current density times water resistivity, hence in the test tank power density equals current density squared times water resistivity.

Nine salmon were tested with the field parallel to their body axis. In none of these fish was there successful conditioning (Table 1).

Magnetic Fields

Only one of 22 eels tested in the three artificial magnetic field situations had a significant Wilcoxon's test value.

Twelve salmon were tested in the same three magnetic field directions. In no case was there any indication of successful conditioning.

Discussion

The migratory routes of American eels and Atlantic salmon almost certainly involve some portions of the major ocean circulation patterns. It would be to a fish's advantage to follow a pathway along which it could utilize as much as possible of the available kinetic energy in the water currents. Since there is strong evidence that passive drift alone is not sufficient to account for the observed movements of some migratory fishes along ocean currents (Royce et al. 1968; Marty 1959), these animals must be able to determine the direction of the water current in order to swim with it. Orientation of a fish in a moving water current without fixed references requires a cue that has some property which is a function of the water direction.

The electric fields generated in ocean water currents moving through the geomagnetic field are perpendicular to the direction of water motion and thus satisfy the requirement. Likewise electric fields generated within the fish's body are perpendicular to the fish's net motion through the

Table 1. Number of American eels and Atlantic salmon showing conditioned cardiac deceleration to weak electric fields at various electric current densities, field intensities, and water resistivities. (⊥ = field applied perpendicular to body axis; ∥ = field applied parallel to body axis. Significance criterion is one-tailed Wilcoxon's signed ranks test at 95% level.) Power available per unit volume is given for each combination.

Electric field ($\mu V/cm$)	Water resistivity (*ohm-cm*)		
	4000	400	40
	American eels		
67.00	0.167×10^{-1} μA/cm² $0.11 \times 10^{+1}$ pW/cm³ 6 of 7 ⊥ significant	–	–
6.700	0.167×10^{-2} μA/cm² 0.11×10^{-1} pW/cm³ 6 of 8 ⊥ significant 0 of 4 ∥ significant	–	–
0.670	0.167×10^{-3} μA/cm² 0.11×10^{-3} pW/cm³ 2 of 8 ⊥ significant	0.167×10^{-2} μA/cm² 0.11×10^{-2} pW/cm³ 8 of 8 ⊥ significant 0 of 4 ∥ significant	–
0.067	0.167×10^{-4} μA/cm² 0.11×10^{-5} pW/cm³ 0 of 8 ⊥ significant	0.167×10^{-3} μA/cm² 0.11×10^{-4} pW/cm³ 0 of 8 ⊥ significant	0.167×10^{-2} μA/cm² 0.11×10^{-3} pW/cm³ 3 of 8 ⊥ significant
	Atlantic Salmon		
6.00	0.15×10^{-2} μA/cm² 0.9×10^{-2} pW/cm³ 6 of 10 ⊥ significant 0 of 3 ∥ significant	–	–
0.60	0.15×10^{-3} μA/cm² 0.9×10^{-4} pW/cm³ 5 of 9 ⊥ significant *0 of 3 ∥ significant*	0.15×10^{-2} μA/cm² 0.9×10^{-3} pW/cm³ 5 of 9 ⊥ significant *0 of 3 ∥ significant*	–
0.06	0.15×10^{-4} μA/cm² 0.9×10^{-6} pW/cm³ 3 of 9 ⊥ significant	0.15×10^{-3} μA/cm² 0.9×10^{-5} pW/cm³ 4 of 9 ⊥ significant	0.15×10^{-2} μA/cm² 0.9×10^{-4} pW/cm³ 5 of 9 ⊥ significant

geomagnetic field, though not necessarily perpendicular to the long axis of its body. Interactions between the local electric fields and the ones generated in the fish may provide information as well.

The horizontal electric fields are generated by water motion through the vertical component of the geomagnetic field. In the Northern Hemisphere the electric currents in the moving surface waters flow from right to left facing downstream. However, the polarity is reversed in the slower bottom waters where the return path for the electric current exists. In the Gulf Stream the electric fields range up to at least 0.46 μV/cm for total electric field intensity (von Arx 1962; Rommel and McCleave 1973), 0.16×10^{-1} μA/cm² for electric current density, and 0.6×10^{-2} pW/cm³ for power density (Rommel and MCleave 1973). If a fish is to utilize the geoelectric fields it must be sensitive enough to perceive the fields and to extract some directional information from them.

The present work demonstrates that these two migratory fishes do have sufficient electro-sensitivity to detect naturally occurring electric fields. In addition, these fishes are sensitive to electric fields perpendicular, but not parallel, to their body axis. Thus the sensitivity of American eels and Atlantic salmon is of the proper magnitude and direction for these fishes to detect geoelectric fields when swimming parallel to the water motion but not when swimming perpendicular to it.

The experiments did not distinguish between sensitivity to a dc field itself or to the change in the field at its onset. However, since the fish moves from side to side as it is swimming, a rapid change in the electric field relative to the fish will occur. Roth (1969) nicely demonstrated in the brown bullhead that such motion of a dc field with respect to the fish's body is enough to stimulate what are basically phasic receptors. It has also been suggested by Kalmijn (1971) that sharks and skates may detect changes in the fields by swimming. Roth (1969) showed in anoperant conditioning experiment that the brown bullhead is able to distinguish dc field polarity. Our experiments were not designed to determine if the fishes are sensitive to polarity of the fields and hence are able to determine upstream from downstream.

TABLE 2. Responses of American eels to electric current density of 0.167×10^{-2} μA/cm² in waters of resistivity 4000 and 400 ohm-cm applied perpendicular to the body axis. (Significance criterion is one-tailed Wilcoxon's signed ranks test between percent change in test beat mean and control beat mean at 95% (*) and 99% (**) level.)

No. of trials	% Change in first control beat Mean ± SD	% Change in first test beat Mean ± SD	% Change in second control beat Mean ± SD	% Change in second test beat Mean ± SD
	4000 ohm-cm water, 6.70 μV/cm			
80	−2.3±13.2	2.7±20.6	−0.5±15.1	7.0±43.0
80	−1.5±16.2	2.2±11.6*	−1.7±16.4	5.9±29.7**
80	−2.1±11.0	2.8± 9.3**	0.4±15.8	6.2±16.3**
80	0.0±19.9	4.2±28.9	−0.4±18.7	7.8±56.2
80	−1.8±13.6	12.2±52.2**	0.8±21.1	5.3±28.6
80	−1.8±16.4	3.7±16.1**	−0.8±16.6	5.2±19.4**
80	−4.8±19.8	2.0±14.9*	−6.6±16.3	2.2±20.1**
80	−5.7±16.3	1.4±16.1**	−4.1±18.6	3.6±17.7**
	400 ohm-cm water, 0.67 μV/cm			
80	−6.0± 9.5	4.6±15.2**	−4.7±10.3	5.7±13.4**
70	−1.0± 9.2	4.4±10.2**	−0.4±10.8	4.6± 9.6**
60	−3.1±16.8	9.8±24.9**	−3.9±18.5	7.4±16.0**
50	−0.4±15.8	12.9±28.5**	0.7±21.3	6.6±22.3
60	−0.6±37.2	10.1±42.2*	1.5±36.7	16.5±51.2
60	2.5±15.2	6.9±16.4	1.1±14.5	9.2±18.7**
80	−1.8±14.9	1.2±16.5	−1.7±20.7	5.4±23.4*
80	−4.4±24.7	5.2±15.4**	−6.8±21.2	14.4±55.4**

It appears from the results (Table 1) that the sensitivity of these fishes to a given electric field intensity is increased with increased salinity. This is consistent with the hypothesis of electroorientation in the sea, since a given water velocity generates the same electric field intensity in fresh or salt water, but the resulting electric current density (and hence the power available) will be greater in saline (conductive) waters.

The reduced number of responses at near threshold power densities is predicted from electrical theory, since some energy must be extracted from a field in order to measure the field. If sufficient power is not available, even relatively high voltages or currents cannot be measured. Generally electrophysiologists have written in terms of sensitivity of biological systems to voltages and voltage gradients without providing enough other electrical information to calculate power available. Since power available is so important at near threshold values, it must be included to give a valid indication of the electrical sensitivity of an organism.

There is considerable variation in the electrical fields near the coast of an ocean. Some electric fields near the shore are much greater than those generated in water currents themselves (P. Manglesdorf personal communication). The configuration of every river and estuary is unique in geography, water flow, water resistivity, bottom resistivity, and magnetic flux density, therefore each of these bodies may have its own electric signature. If the electro-sensitivity of the salmon or eel were acute enough there would be this additional information available to them.

Gleiser and Khodorkovsky (1971) suggested that the European elver is able to sense the presence or absence of the earth's magnetic field. However, their experiments were conducted with only three eels and the details of their methods and results were not clearly stated. Branover et al. (1971) presented somewhat more detail on the experiments, however, these reports simply underline the need for further experimentation.

TABLE 3. Responses of Atlantic salmon to electric current density of 0.15×10^{-2} $\mu A/cm^2$ in waters of resistivity 4000 and 400 ohm-cm applied perpendicular to the body axis. (Significance criterion is one-tailed Wilcoxon's signed ranks test between percent change in test beat mean and control beat mean at 95% (*) and 99% (**) level.)

No. of trials	% Change in first control beat Mean ± SD	% Change in first test beat Mean ± SD	% Change in second control beat Mean ± SD	% Change in second test beat Mean ± SD
	4000 ohm-cm water, 6.0 μV/cm			
80	3.1±21.5	0.7±21.0	−0.3±23.6	1.7±23.6
80	3.8±22.2	0.9±20.5	0.9±20.3	1.6±19.4
80	−0.1± 1.8	0.8± 2.8**	0.0± 1.8	0.4± 2.4
56	−1.0± 5.7	11.6±24.7**	−1.7± 5.4	6.0±17.0**
80	−1.4± 8.9	0.8±15.0*	0.9±10.8	2.7±13.7*
80	−0.1± 3.1	2.4± 7.5**	−0.1± 2.7	1.7± 7.3**
80	−2.1±13.1	6.3±17.3**	−2.2±19.3	6.1±18.6**
48	−4.3±36.1	22.6±48.0**	−0.6±38.1	32.9±52.1**
80	0.2±15.1	5.1±17.4	1.4±16.6	2.2±13.6
80	0.1±11.5	2.5±16.2	−1.1±13.2	2.6±12.4
	400 ohm-cm water, 0.6 μV/cm			
80	0.1±11.1	2.0±13.0	−0.3±12.9	2.2±14.1
80	−1.9±15.2	1.6±15.7	−0.9±14.3	−0.1±10.9
80	−2.6±16.3	1.8±19.7*	1.3±17.8	2.3±19.9
56	−4.0±23.2	23.2±39.0**	0.3±30.9	21.7±44.0**
56	1.2±18.3	3.5±17.9	2.6±15.6	−1.4±15.7
48	−1.1±14.1	8.4±22.8**	−1.0±20.7	9.4±27.7*
56	−0.3± 1.5	0.2± 1.4*	−0.3± 1.5	0.2± 1.4*
72	−0.5±18.9	5.1±24.3*	0.2±17.4	1.7±14.6

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Taxonomy and Ecology of Three New Species of *Monodiamesa* Kieffer, with Keys to Nearctic and Palaearctic Species of the Genus (Diptera: Chironomidae)

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SÆTHER, O. A. 1973. Taxonomy and ecology of three new species of *Monodiamesa* Kieffer, with keys to Nearctic and Palaearctic species of the genus (Diptera: Chironomidae) J. Fish. Res. Board Can. 30: 665–679.

Three Nearctic species of *Monodiamesa* are described: *Monodiamesa depectinata* sp.n. from male imagines, pupa, and fourth- and third-instar larvae; *Monodiamesa tuberculata* sp.n. from imagines, pupae, and fourth- and third-instar larvae; and *Monodiamesa prolilobata* sp.n. from male imagines. A larva possibly belonging to *M. prolilobata* is described. These are the first records and descriptions of imagines of Nearctic species of this genus.

Keys are given for male imagines, pupae, and larvae of the Nearctic and Palaearctic species of the genus.

Monodiamesa depectinata occurs in the Laurentian Great Lakes area and in Lake Winnipeg, Man. It was taken from sandy substrates in the lower littoral and the sublittoral zones of oligotrophic to mesotrophic lakes. *Monodiamesa tuberculata* occurs from the mainland of British Columbia through the prairie provinces and the Northwest Territories to the Laurentian Great Lakes. It was taken from the sublittoral and profundal zones of oligotrophic lakes and in some lakes is probably a glacial relict. *Monodiamesa prolilobata*, from west of the Rocky Mountains, probably covers the combined ecological niches of the two more easterly species.

SÆTHER, O. A. 1973. Taxonomy and ecology of three new species of *Monodiamesa* Kieffer, with keys to Nearctic and Palaearctic species of the genus (Diptera: Chironomidae) J. Fish. Res. Board Can. 30: 665–679.

Nous décrivons trois espèces néarctiques de *Monodiamesa*: *Monodiamesa depectinata* sp.n. à partir d'imagos, de pupes et de larves de quatrième et troisième instars mâles; *Monodiamesa tuberculata* sp.n. à partir d'imagos, de pupes et de larves de quatrième et troisième instars; et *Monodiamesa prolilobata* sp.n. à partir d'imagos mâles. Nous décrivons également une larve, possiblement de *M. prolilobata*. Il s'agit de premières mentions et descriptions d'imagos d'espèces néarctiques de ce genre.

Nous donnons des clés pour l'identification des imagos, pupes et larves mâles des espèces néarctiques et paléarctiques du genre.

Monodiamesa depectinata se rencontre dans la région laurentienne des Grands lacs et dans le lac Winnipeg, Man. On l'a capturé dans les substrats sablonneux des zones littorales inférieures et sublittorales des lacs dont l'état variait d'oligotrophe à mésotrophe. *Monodiamesa tuberculata* est répandu depuis l'intérieur de la Colombie-Britannique jusqu'à la région laurentienne des Grands lacs, en passant par les provinces des Prairies. Il a été capturé dans les zones sublittorales et profondes des lacs oligotrophes et, dans certains lacs, il est probablement un relicat glaciaire. *Monodiamesa prolilobata*, répandu à l'ouest des montagnes Rocheuses, occupe probablement les niches écologiques des deux autres espèces réparties plus à l'est.

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ONE of the major shortcomings in analysis of Nearctic benthic chironomid samples is the lack of knowledge of the specific identities of the typologically more inportant animals. Thus the development of valid lists of indicator communities of lake types such as those known for Palaearctic chironomids (Brundin 1949, 1956b) has hitherto not been possible except for the Arctic ultraoligotrophic communities described by Oliver (1963, 1964).The evaluation of lake typology has to a great extent been based on genera and on the questionable presumption that species with larvae apparently morphologically identical to European species have a similar ecology.

The present paper describes larvae, pupae, and reared imagines of two nearctic species of

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Monodiamesa, the imago of a third species, and a larva possibly belonging to the third species. Although many records of *Monodiamesa* larvae from North America can be found (Johannsen 1937, p. 31; Frantz and Cordone 1966, p. 10; Sæther 1967, p. 197; 1970, p. 6; Brinkhurst et al. 1968, p. 18, 21; Hiltunen 1969a, p. 44; 1969b, p. 128–129; Schneider et al. 1969, p. 83; Sæther and McLean 1972, p. 8),[1] no description or records of adult *Monodiamesa* exist and none of the larvae have been correctly identified to species. [Brundin (1967, p. 367) however, mentions that *Monodiamesa* in North America is represented by two still undescribed species.] The larvae of the three described species are distinct and allow updating of the classification of all previously collected Nearctic larval material. As species of *Monodiamesa* are important members of *Tanytarsus* communities in Northern Europe, the Alps, Japan, and Chile (Brundin 1956b), the typological importance of the described species is obvious.

Methods

The mounting procedure used is outlined by Sæther (1969, p. 1). The general terminology follows Sæther (1971). Characters not mentioned there and measurements and ratios follow Schlee (1966) with the additions and modifications given by Sæther (1969). The terminology of sensillae on antennae and palps follows Serra-Tosio (1970). A number in parentheses following the measurements gives the number of specimens measured (*n*). For some larval measurements the standard deviation (SD) is given.

Holotypes, the allotype of *M. tuberculata*, and, if sufficient material is available, some paratypes have been deposited in the Canadian National Collection (CNC) in Ottawa. Remaining paratypes have been returned to the lender or are retained at the collection of the Freshwater Institute, Winnipeg.

Monodiamesa depectinata sp. n.

The male imago is characterized by the lack of a hind tibial comb; an AR of about 2.4; absence of parascutellars; R_{4+5} without setae; leg ratios of about 0.85, 0.49, and 0.58, and SV_2 about 3.8; anal point with a naked apical spine, and with about 25–33 setae at base; endomere only slightly longer than claspette and about as long as spine of aedagal lobe; and one seta on each of two tubercles near spine of aedagal lobe distad of spine.

The pupa has a relatively short and narrow horn (about 0.55 by 0.06 mm), simple L-setae, and no fringe of setae on IV–VIII.

The larva has very large and broad ventromental plates, each covering or nearly covering a head capsule seta, and with a more or less strong lateral concavity. The distance from apex of lower mental tooth to apex of ventromentum is 0.9–1.2 times the width of a flattened mentum measured from apices of lower mental teeth.

Male Imago (n = 1, Except When Stated Otherwise)

Length 5.48–6.31 mm (2). Wing length 2.56 mm. Total length/wing length: 2.14. Wing length/profemur: 2.47. Thorax blackish-brown; halteres dusky-yellowish white; remaining parts fuscous.

Antenna — Pedicel length/width (microns): 144/200. Antennal groove reaching fourth flagellomere. Flagellomeres 1–2 with sensilla chaeticae. Flagellomeres length/width (microns): 88/58, 30/59, 27/54, 30/50, 26/49, 30/46, 28/46, 27/45, 30/44, 28/42, 32/40, 32/41, 938/43. AR = 2.35–2.44(2).

Head — Temporal setae 13. Postorbitals 6, outer verticals 4, inner verticals 3. Clypeus with 3–5(2) setae. Cibarial pump, tentorium, and cardostipes as in Fig. 2B. Tentorium 230 μ long, maximum width 52 μ, width anterior of posterior tentorial pit 14 μ, distance from apex to posterior tentorial pit 58 μ. Cardostipes 170 μ long, 62 μ wide. Palp lengths in microns: 49, 80, 147, 102, 201. Palp with two sensilla chaeticae at apical sensilla coeloconicae of third segment.

Thorax — Antepronotum with 6–7(2) setae. Humeral pit indistinct, about as in *Monodiamesa tuberculata* sp.n. (Fig. 1 A). Dorsocentrals 11, prealars 4–6(2), parascutellars absent. Scutellum with 12 transverse biserial setae.

Wing — Wing venation, shape, and punctation of normal *Monodiamesa* type. VR = 0.97. Extended part of costa 80 μ long. Basal vein with 2–3(2) setae; R, 8–10(2) setae; R_1, 1–4(2) setae; R_{4+5} without setae. Squama with 23–27(2) setae. Sensilla campaniformis 17–18(2) at base of basal vein, 3(2) below setae of basal vein, 10–11(2) at apex of basal vein, 2(2) ventrally at base of R_1, 1 about 150 μ from base of R_1, and 1 about 30 μ from base of R_{2+3}.

Legs — Spur of front tibia 81 μ long, spurs of middle tibia 58 μ and 64 μ long, of hind tibia 65 μ and 84 μ. Comb absent (Fig. 2D). Tarsal pseudospurs present on ta_1 and ta_2 of p_2 and p_3 measuring 34–46 μ in length. Lengths (in microns) and proportions of legs:

	fe	ti	ta_1	ta_2	ta_3	ta_4
p_1	1036	1282	1092	546	368	233
p_2	1030	1159	570	343	239	165
p_3	1104	1392	810	460	343	202

	ta_5	LR	BV	SV	BR
p_1	141	0.85	2.65	2.92	3.13
p_2	123	0.49	3.17	3.84	2.63
p_3	135	0.58	2.90	3.08	3.42

[1] Roback (1957, p. 54) mentions a species as "*Prodiamesa* nr. *bathyphila* (Kieff.)" This species does not belong in Prodiamesini, but in *Pseudodiamesa* or a related genus.

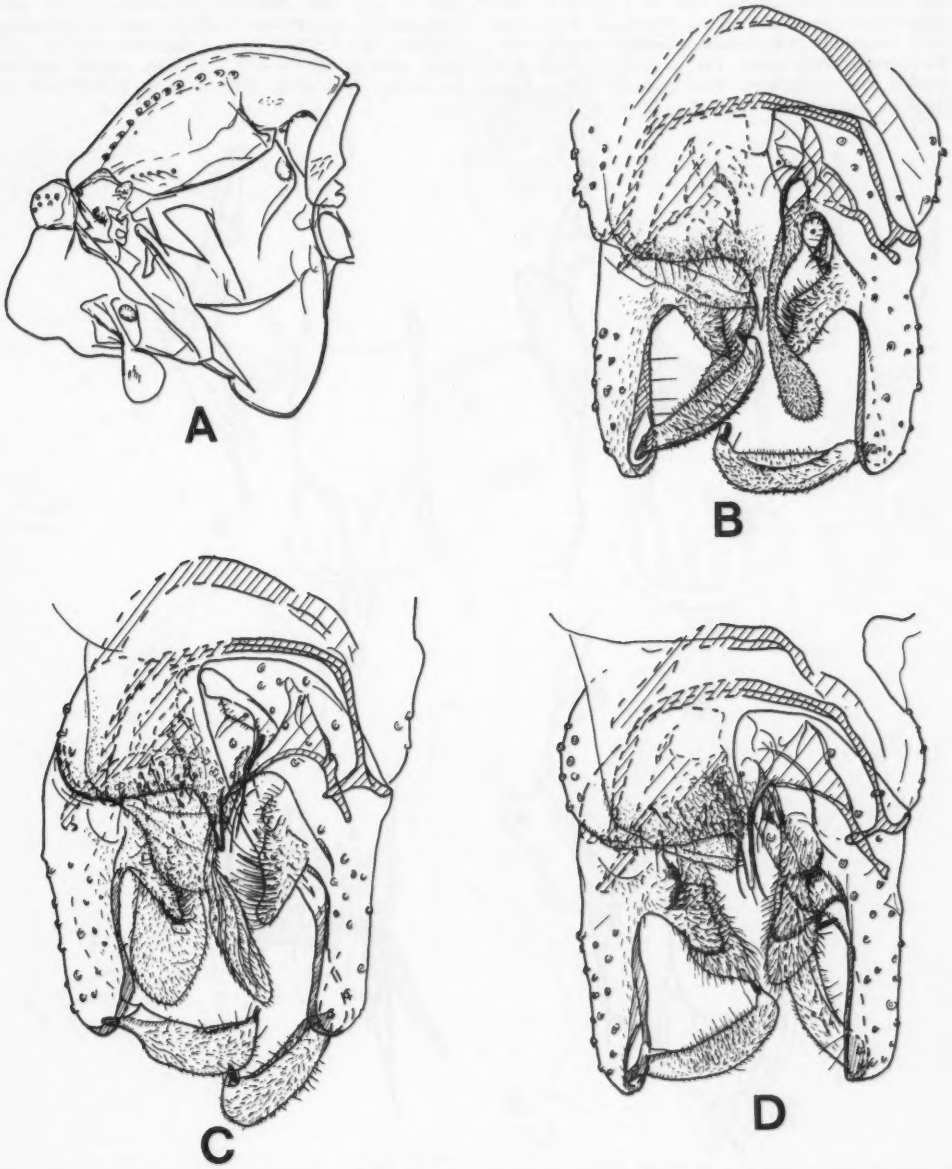

FIG. 1. *Monodiamesa* spp. A, *M. tuberculata* sp.n., thorax, lateral aspect; B, *M. prolilobata* sp.n., hypopygium; C, *M. tuberculata* sp.n., hypopygium; D, *M. depectinata* sp.n., hypopygium.

Hypopygium (*Fig. 1D*) — Ninth tergum with 25–33(2) setae, laterotergites IX each with 10–11(2) setae. Anal point without setae, but with a 10–14 μ(2) long apical spine. Tergum IX with reticulations basal of anal point. Transverse sternapodeme 180 μ long, sternapodeme without oral projections. Phallapodeme 160 μ long. Spine of aedagal lobe 130–144 μ (2) long. Two setae distal of aedagal lobe 50 μ(2) long, placed on 7–8 μ(2) and 10 μ(2) high tubercles. Endomere 133–142 μ(2) long, densely covered with relatively long, but fine setae. Claspette 104–128 μ(2) long. Gonocoxite 342–362 μ(2) long, gonostylus 156–159 μ(2) long. Apical spine of gonostylus 22 μ long. HR = 2.19–2.28(2); HV = 3.51.

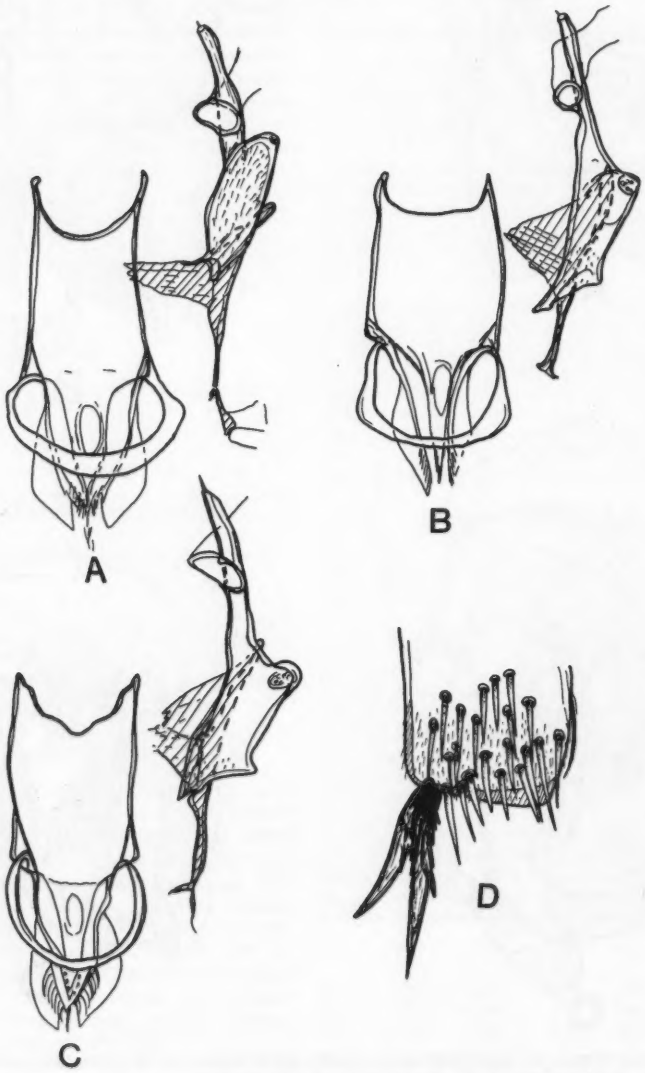

FIG. 2. *Monodiamesa* spp. A–C, cibarial pump, labial lonchus, tormae, tentorium, and cardostipes (cross-hatched); A, *M. tuberculata*, sp.n.; B, *M. depectinata* sp.n.; C, *M. prolilobata* sp.n.; D, *M. depectinata* sp.n., apex of hind tibia.

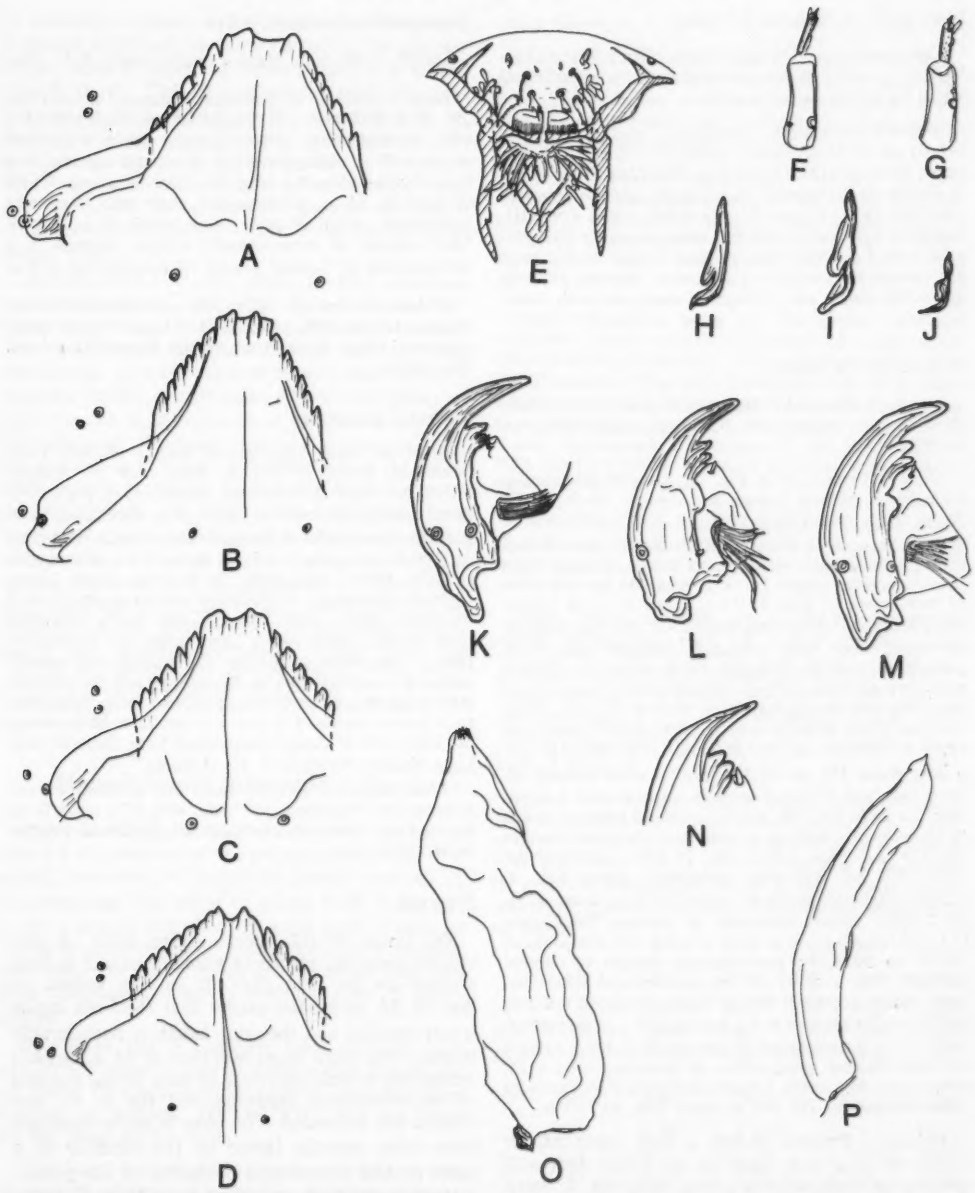

FIG. 3. *Monodiamesa* spp., immatures. A–D, mentum: A, *M. bathyphila* Kieff. from Lake Pluszne, Poland; B, *M. depectinata* sp.n.; C, *Monodiamesa* sp. from Lake Tahoe, California; D, *M. tuberculata* sp.n.; E, *M. tuberculata* sp.n., labrum and palatum; F, *M. depectinata* sp.n., antenna; G, *M. tuberculata* sp.n., antenna; H–J, premandible: H, *M. depectinata* sp.n.; I, *M. tuberculata* sp.n.; J, *Monodiamesa* sp., third instar. K–N, mandible: K, *M. depectinata* sp.n.; L, *Monodiamesa* sp. from Lake Tahoe; M, *M. tuberculata* sp.n.; N, *M. bathyphila* Kieff. from Lake Pluszne, Poland; O–P, thoracic horn of pupa: O, *M. tuberculata* sp.n.; P, *M. depectinata* sp.n.

PUPA (N = 1). LENGTH 6.7 MM

Cephalothorax — Thoracic horn (Fig. 3P) 552 μ long, 61 μ wide, without spines or spinules and of equal width for nearly its full length.

Abdomen — Shagreen, apophyses, and genital sac exactly as in *Monodiamesa bathyphila* (Kieff.) (Thienemann 1919, p. 212–213; 1941, p. 223–224). Pedes spurii A present on sternites IV–VIII, longest spinules about 16 μ long on IV–V, 11 μ on VI, 6 μ on VII, and 3 μ on VIII. Segments I–VI each with 3 L-setae, segments VII–VIII each with 4 L-setae, none divided. Fringe of five setae not present on margins of segments. Segment IX with undivided dorsal seta. Fringe of anal lobe with about 62 setae.

FOURTH INSTAR LARVA

Length 9.82–12.65 mm, mean 11.02, SD 0.80(8). Head capsule length 0.55–0.64 mm, mean 0.61, SD 0.03(8).

Head — Antenna as in Fig. 3F. Lengths of antennal segments in microns (ranges and means): 58–66, 62(7); 24–26, 25(5); 2.0–3.5, 2.5(5); 2.0–2.5, 2.1(5). AR = 2.03–2.25, mean 2.13, SD 0.10(5). Basal antennal segment 21–26 μ wide, mean 23, SD 1.9(7); distance from base to annular organ 14–18 μ, mean 15.8, SD 1.8(6); to basal mark of setae 12–14 μ, mean 13.3, SD 1.0(4); blade at apex 20–32 μ long, mean 27(4), but may possibly be longer when fully extended, acessory blade 9–13 μ long, mean 10.5(4); subapical style of second segment 5–6 μ(3) long. Labrum and palatum as in *M. tuberculata* sp.n. (Fig. 3E). Premandible (Fig. 3H) 70–82 μ long, mean 75.4, SD 5.6(4). Maxilla as in *M. bathyphila* (Thienemann 1919, p. 210–211, fig. 5). Mandible (Fig. 3K) 140–173 μ long, mean 159, SD 11.5(6); with 5 setae serrated on basal side and 2 longer smooth setae in seta interna. Mentum as in Fig. 3B; width measured between apices of lower mental teeth on a completely flattened mentum 106–133 μ, mean 120.0, SD 10.8(8). Ventromental plate with 5–9 fine setae underneath, mean 6.3, SD 1.2(8); maximum width of plate 29–38 μ, mean 32.2, SD 2.8(10); length measured as distance from apex of lower mental tooth to apex of plate 112–144 μ, mean 129.3, SD 10.0(10); posterolateral margin of ventromentum with a more or less pronounced concavity, never evenly rounded; seta of head capsule at posterolateral margin situated 0.5 μ laterad to 7 μ dorsomediad, mean 4.3 μ dorsomediad of margin, SD 2.3(10). Length of ventromental plate/width of mentum 0.92–1.17, mean 1.04, SD 0.08(8). Length of postmentum (mentum plus submentum) 288–362 μ, mean 316, SD 24(10).

Abdomen — Procerci 76–100 μ long, mean 86, SD 9.1(9); 40–50 μ wide, mean 43, SD 3.5(9); each with about seven apical 400–630 μ long, mean 508, SD 85(6), and two lateral setae. Supraanal setae 390–610 μ long, mean 470, SD 87(6). Length of supraanal setae/length of anal setae: 0.76–1.00, mean 0.91 SD 0.09(5). Anal tubules, one pair about 110–150 μ(5), and the other about 140–180 μ(5) long; width about 45–90 μ(5). Posterior parapods 220–280 μlong, mean 248, SD 22(5).

THIRD INSTAR LARVA (N = 1).

Length 3.80 mm. Head capsule length 0.37 mm.

Head — Lengths of antennal segments in microns: 32, 19, 2, 2. AR = 1.39. Basal antennal segment 15 μ wide, distance from base to annular organ 9 μ, blade at apex 23 μ. Subapical style of second segment 4 μ long. Premandible 44 μ long. Mandible 90 μ long. Width of mentum 65 μ. Ventromental plate with four setae underneath, width of plate 18 μ, length 60 μ, seta of head capsule at posterolateral margin situated 3 μ dorsomediad of margin. Length of postmentum 218 μ.

Abdomen — Procerci with 340 μ long apical setae. Supraanal setae 230 μ long. Ratio of length of anal setae/supraanal setae: 0.68. Anal tubules about 124 μ long, 26 μ wide.

MATERIAL EXAMINED

Holotype: male, lighttrap at Biscuit Harbor, Lake Winnipeg, Man., 1/11/71, S. Flam and N. Hooper (CNC No. 12,917). Paratypes: mature male pupa with larval exuvia, three larvae, depth 5 m, shell-sand, Sturgeon Bay near outlet of Dauphin River, Lake Winnipeg, 31/7/69, S. S. Chang; one larva depth 10 m, offshore city of Erie, Penn., Lake Erie, 19–24/11/63, Great Lakes Institute, University of Toronto; one larva, depth 14.5 m, south shore, Lake Erie, 20/7/63, J. K. Hiltunen; three larvae, depth 11 m, sand, south of Manitoulin Island, Lake Huron, 20/7/69, D. Walker; two larvae, depths 4.5 and 16.5 m, at Cheboygan and in Thunder Bay, Lake Huron, 29/9/68 and 21/8/69, J. K. Hiltunen; four larvae, depths 2.5 and 3.5 m, Lake Munuscong, St. Marys River system (connecting Lake Superior and Lake Huron), 30/10/72, J. K. Hiltunen.

Other material: Judging from the drawing of the mentum by Johannsen (1937, fig. 101) of a larva from Green Lake, Wis., this larva in all likelihood belongs to *M. depectinata*.

REMARKS

The imago of this species differs from all previously described species of *Monodiamesa* in lacking a comb on the hind tibia. It is very similar to that of *M. bathyphila* except that the front tarsus is not bearded and the wing length is considerably shorter. The pupa resembles that of *M. bathyphila* except that it lacks the fringe of setae on the margins of the abdominal segments and the L, D, and V-setae are undivided. The larva is easily separated from other nearctic larvae by the presence of a more or less pronounced concavity at the posterolateral margin of the large ventromental plate; the ratio of the length of the plate to the width of the mentum, which is higher than 0.9; and the presence of a head capsule seta hidden by the ventromentum in ventral view. Furthermore, the lower of the two marks representing hyaline setae

or rudiments of them on the basal antennal segment is placed at about the same height as the annular organ, while in figures of other species it is always placed above. The known species with the ventromentum most similar to *M. depectinata* is *M. bathyphila* (Fig. 3A). However, although *M. depectinata* is close to *M. bathyphila*, it does not form its substitute nearctic sister species since *M. bathyphila* is apparently present in North America (represented by a larva from South Indian Lake, Man.).

Brundin (1947, p. 46; 1949, p. 828) mentions that the antepronotum in *M. bathyphila* (as "*M. nigra* sp.n.") and *M. ekmani* Brund. is naked. The three species described here all have antepronotal setae. However, according to a later work by Brundin (1956a, p. 64) there is a lateral group of setae on the antepronotum in all *Prodiamesa* and *Monodiamesa*. Brundin's first two statements must be assumed to be errors, and thus there is no difference in this character among the known species of *Monodiamesa*. All three species mentioned here have some microtrichiae, most numerous in *M. tuberculata*, on the tentorium. This seems to be a unique character and is apparently of value as a generic character. A previously overlooked larval character is the presence of 4–9 fine setae underneath the ventromental plates. These setae are present in all larvae of *Monodiamesa*, but are sometimes very difficult to observe, particularly in the early instars.

Monodiamesa tuberculata sp. n.

The male imago is characterized by an AR of about 1.4; presence of one parascutellar; R_{4+5} with about two setae; a tentorium densely covered by microtrichiae; leg ratios of about 0.69, 0.44, and 0.58; a tergum with about 27 setae, eight of them on the anal point; spine of aedagal lobe on a tubercle; and endomere nearly twice as long as the relatively long claspette and nearly reaching apex of gonocoxite.

The pupa has a relatively wide thoracic horn (about 3.7 times as long as wide) ending in a cluster of about 12 small, hyaline spines; some branched L-setae on segment VII and VIII, and no fringe of fine setae on IV–VIII.

The larva has relatively small and narrow ventromental plates, with evenly rounded posterolateral margins and distance to nearest head capsule seta from ventromentum 6–15 μ. Distance from apex of lower mental tooth to apex of ventromentum is 0.5–0.6 times width of a flattened mentum measured between apices of lower mental teeth.

Male Imago (n = 1)

Length 5.18 mm. Wing length 2.55 mm. Total length/wing length: 2.27. Wing length/length of profemur: 2.55. Thorax brown with brownish-black vittae, lower part of preepisternum and postnotum; halteres dusky-yellowish; remaining parts brown.

Antenna — Pedicel length/width (microns): 134/176. Antennal groove reaching fourth flagellomere. Flagellomeres 1–2 with sensilla chaeticae. Flagellomeres length/width (microns): 60/47, 30/49, 30/48, 30/47, 34/47, 33/45, 31/42, 31/41, 33/40, 34/40, 36/37, 42/37, 601/38. AR = 1.40.

Head — Temporal setae 14. Postorbitals 6, outer verticals 4, inner verticals 4. Clypeus with eight setae. Cibarial pump, tentorium, and cardostipes as in Fig. 2A. Tentorium 205 μ long, maximum width 50 μ, width anterior of posterior tentorial pit 18 μ, distance from apex to posterior tentorial pit 60 μ. Cardostipes 170 μ long, 58 μ wide. Palp length in microns: 47, 66, 123, 100, 205. Palp with two sensilla chaeticae at apical sensilla coeloconicae of third segment.

Thorax (*Fig. 1A*) — Antepronotum with seven setae. Humeral pit indistinct. Dorsocentrals 11, prealars 5, parascutellar 1, Scutellum with 18 transverse biserial, partly triserial, setae.

Wing — Wing venation, shape, and punctation of normal *Monodiamesa* type. VR = 0.97. Extended part of costa 78 μ long. Basal vein with three setae; R, 16; R_1, 7; R_{4+5}, 2. Squama with 28 setae. Sensilla campaniformes 15 at base of basal vein, 3 below setae of basal vein, 9 at apex of basal vein, 2 ventrally at base of R, 1 about 116 μ from base of R_1, and 1 about 30 μ from base of R_{2+3}.

Legs — Spur of front tibia 85 μ long; spurs of middle tibia 62 μ and 63 μ, of hind tibia 63 μ and 95 μ. Width at apex of front tibia 59 μ, of middle tibia 62 μ, of hind tibia 72 μ. Comb with 12 setae, shortest seta 34 μ, longest 60 μ. Tarsal pseudospurs, 36–43 μ long, present on ta_1 and ta_2 of p_2 and on ta_1 of p_3. Lengths and proportions of legs:

	fe	ti	ta_1	ta_2	ta_3	ta_4
p_1	895	1055	730	405	343	196
p_2	994	1006	442	276	202	141
p_3	1055	1190	687	405	343	172

	ta_5	LR	BV	SV	BR
p_1	135	0.69	2.48	2.67	3.03
p_2	123	0.44	3.29	4.52	2.65
p_3	141	0.58	2.76	3.27	3.78

Hypopygium (*Fig. 1C*) — Ninth tergum with 27 setae including eight on anal point, some of them in distal third; laterotergites IX each with eight setae. Transverse sternapodeme 180 μ long, sternapodeme without oral projections. Phallapodeme 150 μ long. Spine of aedagal

lobe 90 μ long including a 38 μ long, 7 μ wide tubercle. *Two setae based of aedagal lobe 34 μ long, on 4 μ high* tubercles. Endomere 236 μ long, broad and flattened apically, covered with fine setae, and nearly extending to apex of gonocoxite. Claspette 124 μ long. Gonocoxite 344 μ long, gonostylus 150 μ long. Apical spine of gonostylus 16 μ long. HR = 2.29; HV = 3.46.

Female (Based on 1 Decomposed Mature Pupa)

Antenna with seven flagellomeres. AR = 1.68. Thorax with about six antepronotals, 13 dorsocentrals, five prealars, one parascutellar, and 17 setae on scutellum. Wing with 2 setae on basal vein, about 15 on R, about *6 on R_1, about 2 on R_{4+5}, and possibly over 50 on* squama. Sensilla campaniformes on basal vein 16 at base, 3 below setae, and 13 at apex. Three ovoid seminal capsules about 90 μ by 80 μ with fine but relatively long scattered microtrichiae. Cerci about 240 μ long.

Pupa (n = 1 Except When Stated Otherwise)

Length 6.33–7.29(2).

Cephalothorax — Thoracic horn (Fig. 30) 724 μ long, 220 μ wide, ending in a cluster of about 12 small, transparent spines.

Abdomen — Shagreen, apophyses, and genital sac apparently as in *M. bathyphila* (Thienemann 1919, p. 212–213; 1941, p. 223–224). Pedes spurii A present on sternites IV–VIII, longest spinules about 15 μ long on IV, 8 μ on V, 5 μ on VI, 4 μ on VII, and 2 μ on VIII. Segments I–VI each with three L-setae; segment VII, 4; segment VIII, 4–5; some L-setae on VII and VIII branched. Fringe of fine setae not present on margins of segments. Dorsal seta of segment IX not observed. Fringe of anal lobe with 60–63 (2) setae.

Fourth Instar Larva

Length 8.09–10.60 mm, mean 8.88, SD 0.87(9).
Head capsule length 0.58–0.63 mm, mean 0.60, SD 0.02(10).

Head — Antenna as in Fig. 3G. Lengths of antennal segments in microns (ranges and means): 56–64, 61(10); 22–26, 23(9); 2–3, 3(9); 1–3, 2(9). AR = 2.00–2.31, mean 2.18, SD 0.10(9). Basal segment 20–24 μ wide, mean 23, SD 1.3(10); distance from base to annular organ 17–37 μ, mean 25; SD 6(10); to basal mark of seta 24–46 μ, mean 36; SD 6(10); blade at apex 21–28 μ long, mean 24, SD 3(5); accessory blade 10–16 μ long, mean 12, SD 2(5). Subapical style of second segment 6–9 μ long, mean 7, SD 1(9). Labrum and palatum as in Fig. 3E. Premandible (Fig. 3i) 70–80 μ long, mean 75, SD 4(8). Maxilla as in *M. bathyphila* (Thienemann 1919, p. 210–211, fig. 5). Mandible (Fig. 3M) 150–162 μ long, mean 157, SD 5.5(4); in seta interna: five setae serrated on basad side and two smooth, longer setae. Mentum as in Fig. 3D. Width of mentum measured between apices of lower mental teeth on a completely flattened mentum 122–140 μ, mean 130, SD 6.5(5). Ventromental plates with 3–6 very fine setae underneath, mean 4.5, SD 1.04 (11); maximal width of plates 14–19 μ, mean 15, SD 1.6(11); length measured as distance from apex of lower mental tooth to apex of ventromental plate on a flattened mentum 66–86 μ, mean 78, SD 6.1(11); posterolateral margin of ventromentum evenly rounded, 6–15 μ from nearest seta of head capsule, mean 9, SD 2.9(11). Length of ventromental plate/width of mentum: 0.49–0.63, mean 0.57, SD 0.06(5). Length of postmentum 296–357 μ, mean 330, SD 18(11).

Abdomen — Procerci 62–100 μ long, mean 83, SD 12(8), 38–56 μ wide, mean 45, SD 6(8); each with about seven apical, 660–810 μ long, mean 712, SD 47(8), and two lateral setae. Supraanal setae 475–550 μ long, mean 500, SD 27(7). Length of supraanal setae/length of anal setae: 0.68–0.83, mean 0.72, SD 0.05(7). Anal tubules, one pair about 120–190 μ(9), and the other about 140–190 μ(8) long; width about 55–80 μ(9). Posterior parapods 250–300 μ long, mean 274, SD 16(8).

Third Instar Larva

Length 5.20–6.99 mm, mean 6.31, SD 0.53(10).
Head capsule length 0.36–0.39 mm, mean 0.37, SD 0.01(10).

Head — Lengths of antennal segments in microns (ranges and means, *n* = 9): 29–33, 31; 16–20, 18; 2–2.5, 2.1; 2–3, 2.3. AR = 1.30–1.45, mean 1.37, SD 0.05(9). Basal segment 13–16 μ wide, mean 14.7, SD 1.0(9); distance from base to annular organ 11–16 μ, mean 13, SD 1.9(5), to basal mark of seta 16–20 μ, mean 18, SD 1.5(5); blade at apex 18–27 μ long, mean 23, SD 3.5(6), accessory blade 10–13 μ long, mean 11.5, SD 1.3(4). Premandible 50–56 μ long, mean 54(3). Mandible 94–125 μ long, mean 112, SD 13(4). Mental width 69–84 μ, mean 78, SD 6(5). Ventromental plate with 4(2) setae underneath; maximum width 9–11 μ, mean 10, SD 0.7(9); length 35–56 μ, mean 44, SD 7(8); seta of head capsule 6–12 μ, mean 8, SD 2.1(10) from margin of ventromentum. Length of ventromental plate/width of mentum: 0.42–0.61, mean 0.52, SD 0.07(5). Length of postmentum 200–218 μ, mean 208, SD 6(10).

Abdomen — Procerci 50–60 μ long, mean 53, SD 3.3(9); 24–28 μ wide, mean 26, SD 1.3(9); each with apical setae 340–440 μ long, mean 401, SD 34(7). Length of supraanal setae: 300–380 μ long, mean 330, SD 27(7), length of anal setae: 0.73–0.94, mean 0.83, SD 0.09(7). Anal tubules: one pair about 90–120 μ long, mean 111(7); other pair about 120–170 μ long, mean 144(7); width about 35–58 μ (7). Posterior parapods 180–200 μ long, mean 192, SD 7(8).

Material Examined

Holotype: male with pupal and larval exuviae, depth 12 m, fine sand and silt, Waukegan, Lake Michigan, collected 15/8/72, emerged 20/8/72, C. Kondrat (CNC No. 12,919). Allotype: mature female pupa reared from larva, depth 6m, fine sand, near Waukegan, Lake Michigan, 13/4/72, C. Kondrat. Paratypes: 11 fourth

instar larvae, 10 third instar larvae, depths 3 and 12 m, fine sand and clay, near Zion and Waukegan, Lake Michigan, 9/8/71 and 15/5/72, C. Kondrat.

Other material (all larvae) — Fifteen in fourth instar, 15 in third, 2 in second, depths 7–17 m, northern third of northern basin, Lake Winnipeg, 7/6-28/10/69, S. S. Chang. 1 in fourth instar, depth 18.5 m, Lake Ontario, 12/9/64, as "*Prodiamesa* cf. *bathyphila*" in Hiltunen (1969a), J. K. Hiltunen; 3 in fourth instar, depths 19–20 m, eastern parts, Lake Erie, 28/9–24/11/63, Great Lakes Institute, University of Toronto; 5 in fourth instar, depths 26–82 m, Georgian Bay, Lake Huron, 10–23/8/64, Great Lakes Institute, Univ. of Toronto; 2 in fourth instar, depths 148–167 m, mostly clay and silt, central part, Lake Huron, 15–17/7/69, D. Walker; 1 in third instar, depth 5.5 m, southwest shore, Lake Michigan, 23/9/62, J. K. Hiltunen; 1 in fourth instar and 1 in third, depth 10 m, 800 m from shore, organic debris, 4 in fourth and 1 in third, depth 15 m, 2050 m from shore, silty clay, transect out from Gary, Ind., Lake Michigan, 4/10/71, S. H. Gurney; 1 in fourth instar and 10 in third, depth 10 m, 840 m from shore, organic debris, 3 in fourth and 1 in third, depth 18 m, 8700 m from shore, organic debris, transect out from Burns Ditch, Ind., Lake Michigan, 3 and 25/10/71, S. H. Gurney; 1 in third instar and one in second, depth 10 m, 590 m from shore, silty sand, 6 in third, depth 15 m, 1020 m from shore, organic debris, 1 in third, depth 18 m, 14,160 m from shore, silty sand, transect out from Michigan City, Ind., Lake Michigan, 2/6, 22/6, and 30/7/71, S. H. Gurney; 5 in fourth instar and 2 in third, depth 28 m, Apostle Island region, Lake Superior, as "*Prodiamesa* cf. *bathyphila*" in Hiltunen (1969b), 2/8/59, 26/9/60, 2/6/61, 4/4 and 4/6/62, J. K. Hiltunen; 1 in second instar, depth 13.5 m, Kalamalka Lake, B. C., as "*Monodiamesa* f.l. *bathyphila*" in Sæther and McLean (1972), 11/6/71, J. F. Flannagan and O. A. Sæther; 1 in third instar, depth 6 m, Keyhole Lake, Mackenzie District, N.W.T., as "*Monodiamesa* cf. *ekmani*" in Sæther (1967), 6/11/63, K. Elgmork.

Remarks

The imago of this species is nearly identical to that of *M. nitida* (Kieff.) (Reiss 1968, fig. 4). The main differences are the smaller size of *M. tuberculata*, the presence of setae in the middle of the anal point, and the endomeres that nearly reach the apices of the gonocoxites. The pupa seems to be unique among *Monodiamesa* in having a cluster of hyaline spines at the apex of the thoracic horn and in having branched L-setae without at the same time having a fringe of setae on the margins of the segments. The larva is easily separated from those of the other two nearctic species as well as from *M. bathyphila* by means of the relatively short and narrow ventromental plates. It is, however, not unlikely that the larvae of *M. nitida*, *M. ekmani*, or *M. alpicola* Brund may have a similar ventromentum. The larvae of the last three species are known (Thienemann 1941, p. 222, footnote: *M. nitida* according to Brundin 1951, p. 50; Brundin 1951, p. 45, *M. ekmani*, and p. 47, *M. alpicola*) but the essential shape of the ventromentum has not been described. The size of the larval eyes has not been measured on the three species described here as only mounted material was available. Judging from the third instar larva found in Keyhole Lake, N.W.T. (Sæther 1967, p. 197), however, *M. tuberculata* probably has eyes like those of *M. ekmani*. However, the larva from Keyhole Lake has a longer antennal blade than the other larvae examined, and it is not impossible that this larva belongs to a different species.

Monodiamesa tuberculata is the substitute nearctic species of the palaearctic *M. nitida* in the same manner as the following species, *M. prolilobata* sp.n., is a substitute of *M. ekmani*.

Monodiamesa prolilobata sp. n.

The male imago is characterized by an orange colored thorax; an AR of about 1.5–1.7; presence of one parascutellar; R_{4+5} with 3–4 setae; leg ratios of about 0.80, 0.43–0.44, and 0.50–0.54, SV_2 of about 4.4; tergum IX with 22–28 setae including 8–10 on anal point; endomere slender, extended, apically rounded, more than twice as long as claspette; and two setae on tubercles at spine of aedagal lobe basad of spine.

The pupa is unknown.

The larvae may possibly be identical to that of *Monodiamesa* sp. described below.

Male Imago (n = 2, Except When Stated Otherwise)

Length 4.29–5.30 mm. Wing length 2.49–2.89 mm. Total length/wing length: 1.72–1.84. Wing length/length of profemur: 2.45–2.48. Thorax orange colored with brown vittae; postnotum and abdomen dark brown; halteres whitish; legs brownish.

Antenna — Pedicel length/width (microns): 150/198(1). Antennal groove reaching fourth flagellomere. Flagellomeres 1–2 with sensilla chaeticae. Flagellomeres not oblique with the exception of the twelfth. Flagellomeres length/width (means in microns): 82/56, 31/53, 35/49, 37/48, 34/48, 34/48, 35/46, 34/45, 34/42, 36/41, 35/39, 38/39, 785/40. AR = 1.53–1.74.

Head — Temporal setae 10–13. Postorbitals 4–5, outer verticals 3–4, inner verticals 3–4. Clypeus with 2–8 setae. Cibarial pump, tentorium, and cardostipes as in Fig. 2C. Anterior margin of cibarial pump deeper than normal, excavated stepwise. Tentorium 260 μ(1) long, maximum width 58 μ(1), width anterior of posterior tentorial pit 15 μ(1), distance from apex to posterior tentorial pit 60 μ(1). Cardostipes 204 μ(1) long, about 50 μ(1) wide. Palp lengths in microns: 44–45, 64–84, 134–172, 120–158, 214–257. Palp with two sensilla chaeticae at apical sensilla coeloconicae of third segment.

Thorax — Antepronotum with 5–6 setae. Humeral pit indistinct. Dorsocentrals 9–12, prealars 3–4, parascutellar 1. Scutellum with 13–18 transverse biserial setae.

Wing — Wing venation, shape, and punctation of normal *Monodiamesa* type. VR = 1.00. Extended part of costa 60–70 μ long. Basal vein with 2–3 setae; R, 10–12; R_1, 5–6; R_{4+5}, 3–4. Squama with 30 setae. Sensilla campaniformes 10–11 at base of basal vein, 2–4 below setae of basal vein, 10 at apex of basal vein, 2 ventrally at base of R_1, 1 at 76–100 μ from base on R_1, and at 40–60 μ from base on R_{2+3}.

Legs — Spur of front tibia 71–76 μ long; spurs of middle tibia 44–53 and 48–59 μ, of hind tibia 55–60 μ and 93–97 μ. Width at apex of front tibia 52–62 μ, of middle tibia 46–57 μ, of hind tibia 68–76, μ. Comb with 13–14 setae, shortest seta 37 μ long (1), longest 70 μ(1). Tarsal pseudospurs, 31–48 μ long, present on ta_1 and ta_2 of p_2 and on ta_1 of p_3. Lengths (in microns) and proportions of legs:

	fe	ti	ta_1
p_1	1006–1178	1312–1588	1055–1270
p_2	1067–1312	1227–1460	540–632
p_3	1141–1374	1497–1725	810–871

	ta_2	ta_3	ta_4	ta_5
p_1	527–626	343–405	209–258	135–153
p_2	350–380	245–307	153–196	123–135
p_3	491–558	343–405	190–215	135–153

	LR	BV	SV	BR(1)
p_1	0.80	2.78–2.80	2.18–2.20	≃3.4
p_2	0.43–0.44	3.25–3.34	4.25–4.39	≃2.7
p_3	0.50–0.54	2.97–2.98	3.26–3.56	≃3.7

Hypopygium (*Fig. 1B*) — Ninth tergum with 22–28 setae including 8–10 on anal point; laterotergites IX each with 10–15 setae. Transverse sternapodeme 160–182 μ long, sternapodeme without oral projections. Phallapodeme 140 μ long. Spine of aedagal lobe 95–108 μ long. Two setae basad of aedagal lobe 42–44 μ(1) long. Endomere 170–220 μ long, narrow, densely covered with fine setae. Claspette 81–90 μ long. Gonocoxite 298–336 μ long, gonostylus 136–152 u long. Apical spine of gonostylus 18 μ(1) long. HR = 2.19–2.21; HV = 3.16–3.49.

Pupa

Not known.

Larva

Possibly identical to that of *Monodiamesa* sp. described below.

Material Examined

Holotype: male, at hut, Marion Lake, B.C., 18/8/65, A. L. Hamilton (CNC No. 12,918). Paratype: male, 19/8/65, otherwise as for holotype.

Remarks

M. prolilobata is very closely related to *M. ekmani* Brund. (Brundin 1949, p. 828–830, fig. 197–198). The main differences are in the number of setae on tergum IX (37 drawn in Brundin's figure of *M. ekmani*, 22–28 in *M. prolilobata*), the shape of the anal point, and, particularly, the shape of the endomere. *M. ekmani* also has obliquely truncated flagellomeres, a slightly higher antennal ratio, and, probably, higher bristle ratios, but the differences in these characters may not be larger than can be expected of infrasubspecific variations.

M. prolilobata is the nearctic sister species of *M. ekmani* from northern Europe. Two other sister species are *M. alpicola* [Brundin 1951, p. 46–48, fig. 22–23) from the Alps and a species from southern Chile (Brundin 1956a, p. 65; 1956b, p. 216–217 as "*M. chilensis* sp. n."; 1967, p. 367, as "*M. patagonica* Brund. (in litt.)"]. *M. ekmani* Brund. seems to be intermediate morphologically between *M. alpicola* and *M. prolilobata*. These four sister species form an excellent example of geographic substitutes.

Monodiamesa sp.

This larva, possibly belonging to *M. prolilobata*, is characterized by medium-sized ventromental plates with evenly rounded posterolateral margins, covering or nearly covering a head capsule seta. The distance from the apex of the lower mental tooth to that of the ventrementum is 0.6–0.7 times the width of a flattened mentum measured between the apices of the lower mental teeth.

Fourth Instar Larva (n = 2 Except When Stated Otherwise)

Length 7.84–10.40 mm. Head capsule length 0.54 mm (1).

Head — Antenna as in *M. tuberculata* (Fig. 3G). Lengths of antennal segments in microns: 62, 24–26, 2(1), 2(1). AR = 2.07(1). Basal segment 28 μ wide; distance from base to annular organ 24–31 μ, to basal mark of seta 32–42 μ; blade at apex 32 μ(1) long, accessory blade 11 μ(1) long. Subapical style of second segment 5 μ(1) long. Labrum and palatum as in *M. tuberculata* (Fig. 3E). Premandible about as in third instar (Fig. 3J). Maxilla as in *M. bathyphila* (Thienemann 1919 p. 210–211, fig. 5). Mandible (Fig. 3L) 150–152 μ long, with five setae serrated on basal side and two smooth longer setae in seta interna. Mentum as in Fig. 3C. Width of mentum measured between apices of lower mental teeth on a completely flattened mentum 120–125 μ. Ventromental plate with 4(1) fine setae underneath; maximum width of plate 19–23 μ; length measured as distance from lower mental tooth to apex of plate on

a flattened mentum 82–89 μ; posterolateral margin evenly rounded, 2 μ mesad to 1 μ ventrolaterad of nearest seta of head capsule. Ratio of length of ventromental plate to width of mentum; 0.66–0.74. Length of postmentum 320 μ(1).

Abdomen — Procerci 80–88 μ long, 48–50 μ wide, each with seven apical setae about 500 μ long and two lateral setae. Supraanal setae not measurable. Anal tubules: one pair 120–130 μ long and the other about 180 μ; width about 40–70 μ. Posterior parapods about 300 μ(1) long.

THIRD INSTAR LARVAE (N = 2 EXCEPT WHEN STATED OTHERWISE)

Length 5.69–6.41 mm. Head capsule length 0.32–0.33 mm.

Head — Lengths of antennal segments in microns: 27–29, 20–22, 2, 2. AR = 1.12–1.13. Basal antennal segment 13–14 μ wide; distance from base to annular organ 10–16 μ; blade at apex not measurable; accessory blade 12 μ(1) long. Subapical style of second segment 4–5 μ long. Premandible (Fig. 3J) 42 μ(1) long. Mandible 80 μ(1) long. Width of mentum 68 μ(1). Ventromental plate with 4(1) setae underneath; 13–14 μ wide, 50 μ long; posterolateral margin 1–2 μ ventrolaterad of nearest seta. Length of ventromental plate/width of mentum: 0.74(1).

Abdomen — Procerci 50 μ(1) long, 20 μ(1) wide, with apical setae about 380 μ(1) long. Supraanal setae about 340 μ(1) long. Length of supraanal setae/length of anal setae about 0.9(1). Anal tubules 80–100 μ long, 30–40 μ wide. Posterior parapods 170 μ(1) long.

MATERIAL EXAMINED

One larva, depth 1.5 m, sand, Westbank, Okanagan Lake, B.C., 9/9/69, J. F. Flannagan and O. A. Sæther; two larvae, depth 11.5 m, Skaha Lake, B. C., 12/5/71, J. F. Flannagan and O. A. Sæther; two larvae, depths 2 and 3.2 m, sand and artificial substrate, Lake Tahoe, Calif., 26/4/66 and 24/8/67, collection of W. T. Mason.

REMARKS

To associate the male imago of *M. prolilobata* with the larvae from other localities would of course be highly tentative. However, the occurrence of the larva in three localities west of the Rockies with no finds in benthic samples from many localities east of the mountains indicates that the larva belongs to a western species. *M. prolilobata* is possibly also restricted to the Pacific coast, and it is at least not unlikely that the larvae described belong to this new species.

Except for smaller size, the larva possibly belonging to *M. proliolbata* more nearly resembles the larva of *M. bathyphila* (the larva of *M. ekmani* is inadequately described) than do those of the other two species. The differences between *Monodiamesa* sp. and *Monodiamesa bathyphila* can be summed up as follows:

	Monodiamesa sp.	*M. bathyphila*
Length of head capsule(*mm*)	ca 54	0.65–0.75
Length of postmentum(μ)	ca 320	380–386
No. of setae underneath ventromentum	ca 4(somewhat weak)	ca 9
Ventromental plate length/ dorsomentum width	0.66–0.74	0.82–0.83

Key to Males of Nearctic and Polaearctic Species of *Monodiamesa*

1. Hind tibia without comb, parascutellars absent, AR about 2.4, endomere only slightly longer than claspette, front tarsus at most slightly bearded .....*M. depectinata* sp.n. (Nearctic)

   Hind tibia with comb, one parascutellar usually present, front tarsus with or without beard.....2

2. Endomere only slightly longer than claspette, AR about 3, front tarsus strongly bearded.....*M. bathyphila* (Kieff.) (Holarctic)[2]

   Endomere much longer than claspette, AR about 2 or lower, front tarsus at most slightly bearded...3

3. Spine of aedagal lobe on tubercle, inner margin of gonostylus more or less concave, front tarsus not bearded.....4

   Spine of aedagal lobe not on a tubercle, inner margins of gonostylus partly straight or convex, front tarsus not or only slightly bearded.....5

4. Endomere nearly reaching apex of gonocoxite, anal point with setae in apical half, tentorium covered by microtrichiae.....*M. tuberculata* sp.n. (Nearctic)

   Endomere clearly not reaching apex of gonocoxite, anal point with setae only at base.....*M. nitida* (Kieff.) (Palaearctic)

5. Maximum width of the slender, apically rounded, endomere not larger than maximum width of the claspette; anal point proper with 8–10 setae: AR = 1.5–1.7.....*M. prolilobata* sp.n. (Nearctic)

   Maximum width of endomere about 1.5–2 times as wide as maximum width of claspette; anal point

[2]There is some doubt whether *M. bathyphila* is Holarctic. Larvae from South Indian Lake, Man., however, are apparently identical with the Palaearctic larvae of *M. bathyphila*. If these larvae do not belong to *M. bathyphila* they have to belong to another, new Nearctic species.

proper at most with 4–5 setae at base, AR about 2.0.....6

6. Endomere with a relatively wide base, apically rounded, and about twice as wide as claspette; front tarsus not bearded.....*M. alpicola* Brund. (Palaearctic)

Endomere with a narrow base, apically footshaped, and about one and one-half times as wide as claspette; front tarsus slightly bearded.....*M. ekmani* Brund. (Palaearctic).

Key to Known Pupae of Nearctic and Palaearctic Species of *Monodiamesa*

1. Margin of abdominal segments IV–VIII with fringe of fine setae, L-setae of VII and VIII branched.....*M. bathyphila* Kieff. (Holarctic)[3]

Margin of abdominal segments IV–VIII without fringe of fine setae, L-setae of VII and VIII simple or branched.....2

2. Thoracic horn with cluster of about 12 small, apical spines; some L-setae of VII and VII branched.....*M. tuberculata* sp.n. (Nearctic)

Thoracic horn without apical spines, L-setae unbranched.....3

3. Thoracic horn short (about 0.6 mm), and slender (about 9 times as long as wide).....*M. depectinata* sp.n. (Nearctic)

Thoracic horn longer and broader.....4

4. Thoracic horn conspicuously broad at base, less than 3 times as long as wide.....*M. alpicola* Brund. (Palaearctic)

Thoracic horn less broad at base, about 3.3–3.6 times as long as wide.....*M. ekmani* Brund. (Palaearctic)

[The pupa of *M. nitida* seems to be most similar to *M. ekmani* according to Thienemann (1941 p. 222, footnote, cfr. Brundin, 1951 p. 50).]

Key to Larvae of Nearctic *Monodiamesa*

1. Ventromental plates relatively small and narrow (13–19 μ wide), ratio of length of plates (measured as distance from apex of lower mental tooth to apex of ventromental plate on a flattened mentum) to width of mentum (measured as distance between apices of lower mental teeth on a flattened mentum) 0.49–0.63, nearest seta of head capsule situated 6–15 μ laterad of ventromental margin.....*M. tuberculata* sp.n. (Nearctic)

Ventromental plates relatively larger and broader (19–38 μ), ratio of length of plates to width of mentum 0.66:1.17, seta of head capsule situated 2 μ laterad of, to 7 μ dorsomediad of plates....2

2. Ventromental plates conspicuously large and broad (29–38 μ), ratio of length of plates to width of mentum 0.91:1.17, seta of head capsule situated at a more or less pronounced lateral concavity (or at least straight margin) 0.5 μ laterad to 7 μ dorsomediad of margin....*M. depectinata* sp.n. (Nearctic)

Ventromental plates about 19–26 μ wide, ratio of length of plates to width of mentum 0.6:0.8, plates evenly rounded, seta of head capsule 2 μ laterad to 2 μ dorsomediad of margin.....3

3. Larva relatively small (about 8–11 mm), postmentum about 320 μ, ventromental plates 19–23 μ wide, ratio length of plates to width of mentum about 0.66:0.74, about four fine setae underneath ventromentum.....*Monodiamesa* sp. (possibly *M. prolilobata* sp.n.) (Nearctic)

Larva large (about 13–16 mm), postmentum about 380–386 μ, ventromental plates about 24–26 μ wide, ratio of length of plates to width of mentum about 0.82:0.83, about nine distinct setae underneath ventromentum....*M. bathyphila* Kieff. (Holarctic)[4]

[*M. ekmani* can apparently be separated from *M. bathyphila* by means of its larger eye size (Brundin 1951, fig. 20–21). However, the new nearctic species are described on the basis of mounted material.]

Distribution and Ecology

The recognition of three quite distinct larvae, the association between the different stages through rearing of the two eastern species and the tentative association of the third larva and imago have made it possible to examine the species distribution of all present and all previously collected larvae of *Monodiamesa* in North America.

The distribution indicates that *M. depectinata* is an eurythermic species, most commonly found on sandy substrates in the lower littoral and sublittoral zones of oligotrophic to mesotrophic lakes. As for *M. bathyphila* (Brundin 1949 p. 667, 710–712; 1956b, p. 203–206), small populations of *M. depectinata* have been found in some eutrophic lakes indicating that the inhabited environment is not too extreme. The type locality of the larva, Sturgeon Bay, is strongly influenced by the saline effluents of Dauphin River. The bay had a conductivity of about 1000 µmhos, in comparison with about 290 µmhos for the rest of Lake Winnipeg. The sediments consist almost exclusively of shell-sand and sand with very little organic detritus. As in all other parts of the relatively shallow and wind-exposed Lake Winnipeg, the oxygen is close to saturation at all or nearly all times. The water temperature near the bottom was 20 C on July 31, 1969. The water in this bay is very clear and the production apparently low. In Lake Erie the two finds of *M. depectinata* were outside or slightly

[3]See footnote 2.

[4]See footnote 2.

west of the city of Erie, Penn., at depths of 10–14.5 m, while *M. tuberculata* was present at several stations at depths of 19–20 m. In Lake Huron *M. depectinata* was found in the north and northwest at depths of 4.5–16.5 m; in Lake Munuscong, which is part of the river system connecting Lake Huron and Lake Superior, at depths of 2.5–3.5 m. Schneider et al. (1969, p. 83) mention "*M. bathyphila*" from Saginaw Bay, Lake Huron. They divide the bay into three horizontal zones and three depth zones. *Monodiamesa* was most common in the outer deeper zone below 42 ft (12.8 m), but one specimen was found in the depth zone of 0–18 ft (0–5.5 m) and two specimens in the depth zone of 18–42 ft (5.5–12.8 m). Since both *M. depectinata* and *M. tuberculata* are present in Lake Huron, it is difficult to evaluate these findings. However, judging from the distribution in other parts of Lake Huron, most or all of the larvae belong to *M. depectinata*. The larva called *M. bathyphila* by Johannsen (1937, p. 31), collected in the deep waters of Green Lake, Wis., almost certainly belongs to *M. depectinata*. Green Lake is highly productive, but with a heterograde oxygen curve and a large hypolimnion and, therefore, morphometrically oligotrophic. In the first published paper on this lake, Marsh (1892) recorded the presence of *Pontoporeia* and *Mysis*, together with *Monodiamesa* well-known members of moderate oligotrophic communities. Juday (1924) gives a summary of quantitative investigations on the lake, He divides the chironomid fauna into *Tanypus*, *Chironomus*, *Orthocladius*, and *Tanytarsus*. "*Orthocladius*," almost certainly including *M. depectinata*, occurs only down to 10 m except for one occurrence in the 10–20 m zone.

The above-mentioned lakes differ significantly in chemical composition and productivity ranging from oligotrophic to weakly eutrophic (Lake Erie is eutropic in the western part, but mesotrophic to moderately oligotrophic in the eastern part). However, all of them have species such as *Mysis relicta* Lovén, *Pontoporeia affinis* Lindström, and *Limnocalanus marcrurus* Sars as members of their fauna and are well supplied with oxygen at least in some parts of their profundal zones.

M. tuberculata seems to have an ecology conforming to that of a glacial relict. It is most common in the deeper waters of large or deep oligotrophic lakes though it has been found in the littoral zone of Lake Michigan at depths of 3–18 m (samples not having been taken at depths greater than 18 m). It has also been taken at depths of 7–17 m in the northern third of the north basin of Lake Winnipeg, at 18.5 m in Lake Ontario, at 19–20 m in Lake Erie, at 26–82 m in Georgian Bay of Lake Huron, at 148–167 m in Lake Huron, and at 28 m in Lake Superior (probably also at 9–45 m according to Hiltunen 1969b, p. 128–129). In addition, single specimens have been found in Keyhole Lake, N.W.T., a subarctic lake, and at a depth of 13.5 m in the northeastern bay of Kalamalka Lake, B.C. (Sæther and McLean 1972, table 3), an oligotrophic lake. Although bottom temperatures may exceed 18 C at shallow stations both in Lake Winnipeg and in Lake Michigan, these high temperatures last only for a short time. The presence of *M. tuberculata* in the shallower waters of Lake Michigan is in contrast with the distribution in Lake Ontario, Lake Erie, and Lake Huron. However, in the last two lakes there are two species of *Monodiamesa*. The distribution pattern in shallower areas thus may be a result of presence or absence of competition. In Lake Erie it was found at four stations, all in the 19–20 m layer. All the stations fall outside the shaded or dotted areas in fig. 2 of Brinkhurst et al. (1968) and thus outside areas with less than 75% oxygen saturation at the bottom. The species is probably unable to occupy the deeper water because of the O_2-conditions and the shallower water because of competition from *M. depectinata*, the higher temperatures, a combination of both, or other factors. Unless the conditions improve, *M. tuberculata* is likely to disappear both from Lake Ontario and from Lake Erie in the same manner as *L. macrurus* Sars has from Lake Erie (Gannon and Beeton 1971).

If the deepwater specimens of *Monodiamesa* from Lake Tahoe (Frantz and Cordone 1966) belong to the *Monodiamesa* sp. described here, the ecological niche of this species is very similar to that of *M. bathyphila*: the profundal zone of deep oligotrophic lakes as well as the littoral and sublittoral areas of mesotrophic and weakly eutrophic lakes. The western species thus seemingly occupy the ecological niches of the two eastern species combined.

The males of *M. prolilobata* were collected at the shore of Marion Lake (Hamilton 1965, p. 51, as *Prodiamesa* sp.), a small oligotrophic lake in British Columbia. *Monodiamesa* sp. (the larva possibly belonging to *M. prolilobata*) was found in Okanagan and Skaha lakes in B.C. and in Lake Tahoe at the border of California and Nevada. The only specimen from Okanagan Lake was from sand at a depth of 1.5 m outside a sewer outlet at Westbank. Although Okanagan Lake is a typical oligotrophic lake, the station with *Monodiamesa* sp. (Sta. 7) had a faunal composition typical of mesotrophic conditions (Sæther 1970, p. 13). Only two specimens were found in Skaha Lake, both at Sta. 15 at a depth of 11.4 m on

a substrate of gyttja and debris (Sæther and McLean 1972, table 3). During the previous survey no *Monodiamesa* were found in Skaha (Sæther 1970). Skaha Lake, formerly oligotrophic, is rapidly becoming eutrophic because of pollution. It does, however, still possess oligotrophic to mesotrophic conditions concerning oxygen. The two larvae from Lake Tahoe were found on sandy substrate and artificial substrate at depths of 2–3.2 m. W. T.Mason (personal communication) found the larvae at depths of 2–18 m on sandy substrates all around the lake, but never in the less sampled profundal zone. Frantz and Cordone (1966, p. 10), however, mention *M. bathyphila* as widespread below 100 feet. The inconsistency between these findings is most likely due to insufficient sampling. The deepwater *Monodiamesa* may, however, belong to a different species. Lake Tahoe is an ultraoligotrophic lake situated 1897 m above sea level, with a maximum depth of 501 m and a mean depth of 313 m (Kemmerer et al. 1924; Weidlein et al. 1965). The species from Lake Tahoe called "*Metriocnemus* near *lundbecki* (Joh.)" by Frantz and Cordone (1966, p. 10) is unlikely to be near *Parametriocnemus lundbecki* (Joh.), since the genus *Parametriocnemus* does not occur in lakes, but is in all likelihood a *Heterotrissocladius*.

When the three species of *Monodiamesa* described here occur in the littoral and sublittoral areas they all seem to prefer the sandy areas. This is in accordance with the statement by Pagast (1947, p. 587) for other *Monodiamesa*.

Henson (1966, p. 37) considered that, while descriptive biotic surveys were still needed concerning Great Lakes benthos, investigations should be directed toward answering the key questions of "why" and "how" rather than "what." However, of the dozens of publications on Great Lakes benthos, none deals comprehensively with one of the most important groups, the chironomids. Although a few papers have gone to the generic and tentatively to the specific level, only Johnson and Brinkhurst (1971) have attempted through rearing to get valid specific names, and they have confirmed the specific identifications of only 18 of 43 species. Moreover, some of their identifications are doubtful. There are for instance probably at least three nearctic species with larvae like those of *Heterotrissocladius subpilosus* (Kieff.) (see Sæther and McLean 1972) and a comparison between the Palaearctic and Nearctic specimens called *H. subpilosus* still remains to be done. In Great Lakes benthic research, as well as in benthic research in the rest of North America, the key question is still what is present. However, the "whats" can and should go hand in hand with the "whys" and "hows."

Acknowledgments

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NOTES

An Outbreak of Columnaris Disease in White Suckers (*Catostomus commersoni*) in Saskatchewan

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WOBESER, G., AND F. M. ATTON. 1973. An outbreak of columnaris disease in white suckers (*Catostomus commersoni*) in Saskatchewan. J. Fish. Res. Board Can. 30: 681–683.

Columnaris disease, due to *Chondrococcus columnaris*, was diagnosed during a die-off of white suckers (*Catostomus commersoni*) in a lake in Saskatchewan. The pathology of the disease in white suckers is described. This is the first known report of columnaris disease from the prairie region of Canada.

WOBESER, G., AND F. M. ATTON. 1973. An outbreak of columnaris disease in white suckers (*Catostomus commersoni*) in Saskatchewan. J. Fish. Res. Board Can. 30: 681–683.

Les mortalités catastrophiques de catostomes noirs communs (*Catostomus commersoni*) qui se sont produites dans un lac de la Saskatchewan ont été diagnostiquées comme étant le résultat de la maladie du columnaris, causée par *Chondrococcus columnaris*. Les auteurs décrivent les manifestations pathologiques de cette infection chez le catostome noir commun. Il s'agit de la première mention de cette maladie dans la région des Prairies canadiennes.

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COLUMNARIS disease, due to the myxobacterium *Chondrococcus columnaris* and first isolated and described by Ordal and Rucker (1944), is of prime importance as a disease of returning anadromous salmon, but also has been diagnosed in many other species of fish (Pacha and Ordal 1970).

In Canada the disease has been recognized in wild sockeye salmon (*Oncorhynchus nerka*) from two watersheds in British Columbia and in hatchery reared rainbow trout (*Salmo gairdneri*) in Quebec (Canadian Committee on Fish Diseases 1972). Experimental work as well as observations on outbreaks (reviewed by Pacha and Ordal 1970) indicated that "scrapfish" including suckers could harbor the organism and that these fish could serve as a source of infection for salmonids. Documentation of the occurrence of columnaris disease in native fish is important if the disease is to be considered under regulations governing the importation of fish and the control of fish diseases in Canada.

This report describes an outbreak of columnaris disease that occurred in white suckers (*Catostomus commersoni*) in Buffalo Pound Lake, Saskatchewan, during 1972.

Buffalo Pound Lake, located approximately 15 km north of Moose Jaw, Sask., is the headwater lake of the Qu'Appelle River system. This is a eutrophic lake with a surface area of 29.5 km^2 and a mean depth of 3 m. Since 1958 the lake has been linked to the South Saskatchewan River by either a pumping system or a diversion ditch. This has resulted in a decrease in nutrient salts and in the introduction of several species of fish previously not found in the lake (unpublished data). The white sucker is native to the Qu'Appelle drainage system.

On June 2, 1972, mass mortality of white suckers was reported in Buffalo Pound Lake. The following day approximately 300 dead suckers were evident along approximately 2 km of shoreline formed by two highways crossing the north end of the lake. Numerous moribund suckers were present in shallow water in the lee of the highways. The water temperature was 19.4 C. Many anglers were present in the general

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area and approximately 15 northern pike (*Esox lucius*), which they had captured, were examined. None of these fish had grossly visible lesions and no dead fish other than white suckers were observed in the area. A large proportion of the moribund suckers appeared to be blind in one or both eyes.

Materials and methods — Six moribund suckers weighing from .7 to 1.3 kg were captured from shore without the aid of a net, examined, packed on ice in coolers, and taken to the laboratory within 2 hr. In the laboratory, impression smears made from surface lesions, gills, and material aspirated from the eyes were stained with Gram stain, and other smears were examined as wet mounts by phase contrast microscopy. The abdominal wall was incised using flamed instruments, exposing the viscera but leaving the swim bladder covering the kidney. Using flamed forceps, the swim bladder was reflected forward and a hot flamed microbiologic loop was inserted into the anterior kidney. Trypticase soy agar (TSA), blood agar (5% citrated sheep blood) (BA), and Ordal's medium (Ordal and Rucker 1944) (OM) incubated at room temperature (22 C) were used for primary isolation from the kidney. Representative colonies from OM were subcultured on the same media and subcultures from three of the six fish were forwarded to E. J. Ordal, University of Washington, for identification.

Portions of liver, anterior and posterior kidney, spleen, stomach, intestine, gill, eyes, and skin and muscular lesions were immersed in Bouin's fixative for 24 hr, postfixed in 70% alcohol until trimmed, processed by routine methods, sectioned at 5 μ, and stained with haemotoxylin-eosin.

Pathology and diagnosis — All of the fish collected, together with approximately 20 other moribund suckers and the dead fish observed in the lake, had large ulcerative lesions with hemorrhagic borders on the body surface. These were particularly severe on the caudal portion of the body (Fig. 1, 2). The fins were hyperemic with scattered petechial or larger hemorrhages. Two of the six fish collected had turbid material in the anterior chamber of the eye. The gills were hemorrhagic with focal areas of necrosis covered by deposits of thick grey material. Hemorrhage from the gill area occurred when moribund fish were handled. The visceral organs were unremarkable.

Impression smears taken from surface lesions, the anterior chamber of the eye, and the gills contained masses of long, thin, filamentous Gram-negative organisms. Wet mounts from these sites contained masses of long, thin, flexible filamentous rods motile by a gliding motion.

No growth was obtained on TSA or BA, but light growth of a bacterium with a flat filamentous yellow colony was obtained from the anterior kidney of all six fish after 2–5 days incubation on OM. Morphologically and tinctorially this organism corresponded to that seen on the impression smears. Three subcultures of this organism were subsequently identified as *C columnaris* by E. J. Ordal, University of Washington.

The surface lesions observed were similar histopathologically to those reported by Pacha and Ordal (1967) in experimentally infected sockeye salmon, with loss of scales and hemorrhage in the scale pockets. Large numbers of bacteria were present in the ulcerated areas and, in some cases, there was hyaline degeneration of the underlying muscle. There were few or no inflammatory cells present in the lesions.

The lesions in the gills were of a hemorrhagic nature with massive collections of erythrocytes in the interlamellar spaces, desquamation of the epithelium of the secondary lamellae, and thrombosis of both lamellar lacunae and larger vessels of the filaments. Masses of bacteria were present in the desquamated debris and the filaments. Glomerular lesions in the kidney, as described by Pacha and Ordal (1967), were not prominent. The most conspicuous change noted in the visceral organs was a generalized activation of reticuloendothelial (RE) elements. There was degeneration of hematopoietic tissue in the kidney, with hypertrophy and hyperplasia of RE cells in the kidney, spleen, and liver. Ocular lesions present in two of the six fish consisted of retrobulbar edema and retinal detachment with intraocular bacterial growth. The cornea of one of these fish was ulcerated.

Discussion — The gross lesions seen in these suckers were similar to those reported in columnaris disease in other species (reviewed by Pacha and Ordal 1970). The histopathologic lesions were more extensive than those reported in experimental columnaris disease by Pacha and Ordal (1967) and suggested a primary gill and surface infection that may have become septicemic only as a terminal event. The presence of well-advanced surface and gill lesions and systemic infection might indicate a low virulence strain of *C. columnaris* as described by Amend (1970).

Though this is apparently the first report of columnaris disease from the prairie region of Canada, one of us (F.M.A.) has observed similar die-offs of suckers in the spring of the year in other waters in southern Saskatchewan, so it is possible that the disease may be rather widespread. Pacha and Ordal (1970) suggested that "scrap-fish" including suckers may maintain the organism in an inactive state during cold weather. As water temperatures rise, active infection may occur in these

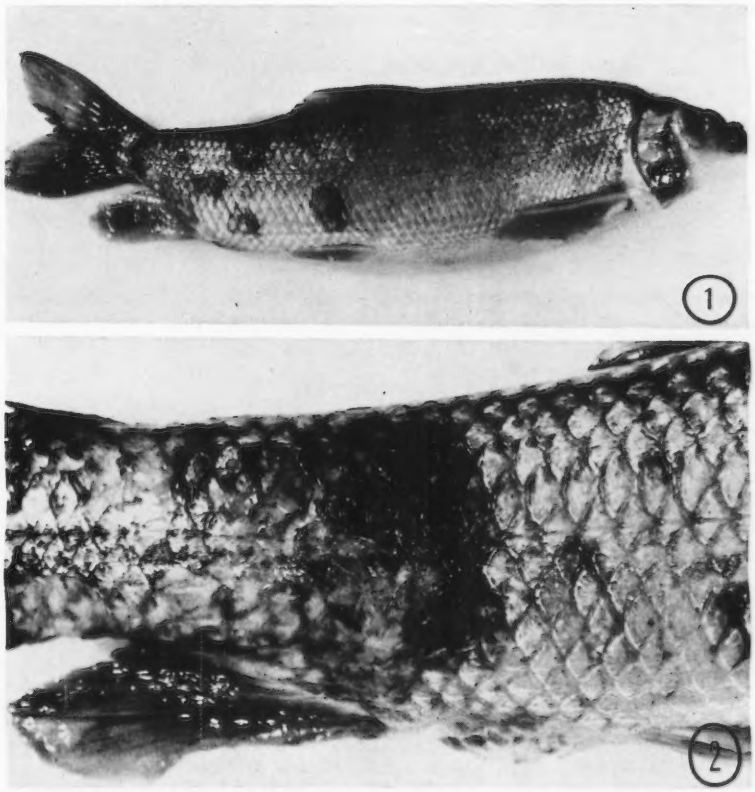

FIG. 1, 2. White suckers (*Catostomus commersoni*) with columnaris disease. (1) Several large ulcerative lesions on body surface and operculum. Also hyperemia and hemorrhages in caudal and anal fins. (2) Ulcerative lesion with hemorrhagic margin and loss of scales.

fish and the disease can then be readily transmitted to other species including salmonids. Further sampling is required to determine the incidence of the disease in native fish in western Canada, particularly if salmonids such as rainbow trout are to be introduced into waters containing such fish. A survey for the presence of agglutinating antibodies against *C. columnaris* as used by Fujihara and Hungate (1972) might be very useful in this regard.

Acknowledgments — The authors are deeply indebted to Prof. E. J. Ordal, Department of Microbiology, School of Medicine, University of Washington, Seattle, Wash., for the identification of the bacterium isolated from these fish.

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First Records of the Stoneroller (*Campostoma anomalum*) and the Blackstripe Topminnow (*Fundulus notatus*) from Canada

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GRUCHY, C. G., R. H. BOWEN, AND I. M. GRUCHY. 1973. First records of the stoneroller (*Campostoma anomalum*) and the blackstripe topminnow (*Fundulus notatus*) from Canada. J. Fish. Res. Board Can. 30: 683–684.

Stoneroller (*Campostoma anomalum*) and blackstripe topminnow (*Fundulus notatus*) were found, respectively, in the Thames and Sydenham rivers of southwestern Ontario. These are new records for Canada.

GRUCHY, C. G., R. H. BOWEN, AND I. M. GRUCHY. 1973. First records of the stoneroller (*Campostoma anomalum*) and the blackstripe topminnow (*Fundulus notatus*) from Canada. J. Fish. Res. Board Can. 30: 683–684.

Capostoma anomalum et *Fundulus notatus* ont été découverts respectivement dans les rivières Thames et Sydenham du sud-ouest de l'Ontario. Il s'agit de premières mentions pour le Canada.

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IN a series of collections of fishes made by two of us (CGG and RHB) in southwestern Ontario in August 1972, we have discovered two species, stoneroller (*Campostoma anomalum*) and blackstripe topminnow (*Fundulus notatus*), not previously recorded from Canadian waters (Scott and Crossman 1969).These collections are now housed in the Ichthyology collection of the National Museum of Natural Sciences (NMNS), Ottawa, with the exception of a series of each of the newly recorded species that was forwarded to the Royal Ontario Museum (ROM), Toronto. Both species

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are known from streams in the Great Lakes drainage in Michigan and Ohio (Hubbs and Lagler 1958; Trautman 1957).

Fundulus notatus was found in two localities in the Sydenham River drainage. Forty-eight specimens were taken in Black Creek, Enniskillen Township, Lambton County, on August 10, 1972. Of these, 38 are catalogued at the NMNS as NMC72-184 and ten are catalogued at the ROM as 28312. Eight additional specimens were caught in the North Sydenham River, Sombra Township, Lambton County, on August 10, 1972, and are catalogued as NMC72-199. *Fundulus notatus* is readily distinguished from *F. diaphanus*, the only sympatric cyprinodontid in Canadian waters, by the presence of a prominent, horizontal, black lateral band that is lacking in *F. diaphanus*. Both sexes of *F. diaphanus* have dark, vertical bars (Hubbs and Lagler 1958). In *F. notatus* these are present in the male only and are less prominent than in *F. diaphanus*.

In our sample of *F. notatus* the number of lateral line scales ranged from 32 to 35 and the number of dorsal rays was eight or nine. In *F. d. diaphanus*, the eastern subspecies, these meristic values were, respectively, 40–55 and 13 or 14 [range 12–15] (Hubbs and Lagler 1958). In *F. notatus* the origin of the dorsal fin is posterior to the origin of the anal, whereas in both *F. diaphanus* and *F. heteroclitus* the converse is true. *F. heteroclitus*, the only other species of *Fundulus* so far known from Canadian waters, occurs only along the Atlantic coast (Leim and Scott 1966) and is not sympatric with *F. notatus*.

Campostoma anomalum was found in Nineteen Creek, a tributary of the Thames River in Blanshard Township, Perth County. A large series of specimens, of which ten were deposited in the ROM (Cat. No. 28311), are catalogued at the NMNS as NMS72-208. This species is readily distinguished from all other Cyprinidae known in Canada by the presence of a prominent cartilaginous ridge located anteriorly on the lower jaw and separated by a groove from the lower lip; the intestine is spirally wound about the gas bladder. Superficially, this minnow somewhat resembles a sucker.

Hubbs and Lagler (1958) recorded three characters that were used to separate the Great Lakes basin subspecies of *C. anomalum*: number of lateral line scales, number of scale rows around the body, and ratio of head length to gape width. On the basis of these three characters, the Thames River drainage specimens appear most similar to the Ohio stoneroller, *C. a. anomalum* (Table 1). On zoogeographic grounds, however, it would seem more reasonable that the Ontario specimens be *C. a. pullum*, for that subspecies is distributed most closely to the shores of lakes Erie and St. Clair in the USA (Trautman 1957). For the time being we do not, therefore, make a subspecific designation for the Ontario specimens.

TABLE 1. Comparison of characteristics used to separate the Great Lakes basin stoneroller subspecies, *Campostoma anomalum oligolepis*, *C. a. anomalum*, and *C. a. pullum* (data from Hubbs and Lagler 1958), with 20 specimens from the Thames River, Ontario.

Subspecies or locality	Scales in lateral line	Scale rows around body	Head length	
			Gape	width
C. a. oligolepis	43–47	31–36	4.3–4.8	
C. a. anomalum	48–51	36–42	3.7–4.4	
C. a. pullum	49–55	39–46	4.6–5.5	
Thames River, Ontario	46–53	38–43	3.8–4.5	

We are undecided as to whether these two species have existed in Canadian waters for many years. Southern Ontario has been fairly thoroughly sampled in the past, and neither of these species has been reported. Trautman (1957) reported an expansion of range for *F. notatus* of which the present appearance in Ontario may be representative. Trautman suggested that the range of *F. diaphanus* in Ohio decreased as waters became more turbid, and the lack of competition recently permitted the turbidity-tolerant *F. notatus* to extend its range. This same combination of factors may have permitted *F. notatus* to recently cross Lake St. Clair to the Sydenham River. On the other hand, *C. anomalum* is not noted for its ability to withstand oxygen-depleted, polluted, or silted habitats (Trautman 1957), and we find it difficult to imagine that this species has only recently crossed Lake St. Clair and penetrated the Thames River to a point above the city of London. We also recognize that these species could have entered Canada illegally as bait or aquarium fishes, but we do not feel this is probable.

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Effect of Sodium Tripolyphosphate on Thaw Drip and Taste of Fillets of Some Freshwater Fish

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The effect of polyphosphate treatment on thaw drip was determined on six freshwater fish species. No relation could be found between state of rigor and thaw drip in control and treated fillets or increase in weight due to dipping. Thaw drip was reduced by an average of 60% as a result of treatment. The difference in weight between control and treated fillets after thawing averaged 5% or more of the initial weight in favor of the treated products. A difference in taste could be detected in yellow walleye (*Stizostedion vitreum*) and in whitefish (*Coregonus clupeaformis*) samples, but preferences were equally divided between treated and untreated samples.

MANOHAR, S. V., D. L. RIGBY, AND L. C. DUGAL. 1973. Effect of sodium tripolyphosphate on thaw drip and taste of fillets of some freshwater fish. J. Fish. Res. Board Can. 30: 685–688.

Les auteurs évaluent, sur six espèces de poissons d'eau douce, les effets du traitement au polyphosphate sur l'exsudat de décongélation. Ils ne trouvent aucune relation entre l'état de rigor et l'exsudat des filets témoins et des filets traités, et l'augmentation de poids à la suite de l'immersion. Le traitement réduit d'environ 60% en moyenne l'exsudat de décongélation. La différence de poids, après décongélation, entre les témoins et les filets traités est en moyenne 5% ou plus du poids initial, en faveur du produit traité. On a pu déceler une différence de goût chez le doré jaune (*Stizostedion vitreum*) et le grand corégone (*Coregonus clupeaformis*), mais les préférences se sont réparties également entre les échantillons traités et non traités.

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THE factors affecting drip and thaw drip in fish were reviewed by Miyauchi (1963); he reported that the amount of drip can vary from species to species and also from fish to fish. Dyer et al. (1964) traced the history of the use of phosphate compounds as water retainers in cheese and in meat products and reported no reduction in drip in cod with the use of phosphate. Dyer (1969) discussed the respective effectiveness of sodium chloride brine and phosphate solutions in controlling drip in fish. MacCallum (1969) stated that each processor should determine the optimum conditions for applying a phosphate dip to his products, depending on the type of market and the species. Now that the freshwater fishing industry has entered the field of processing, information on drip control and its effect on taste becomes pertinent; the present investigation was carried out at the request of the Freshwater Fish Marketing Corporation with headquarters in Winnipeg, because no information was available concerning drip control in freshwater fish.

Methods — Because thaw drip increases with time in chilled storage before freezing (Sutton 1969) samples of uniform freshness were obtained by killing live fish, gutting and cleaning immediately, and storing in ice. Samples of varying state of rigor were secured by removing individual fish at the appropriate time; pre-rigor fish after 4 hr, in-rigor fish after 24–28 hr (depending on species) and post-rigor fish after 5–7 days. On removal from the ice, skinless fillets were obtained from these fish, one fillet going to treatment, the corresponding fillet serving as a control. Eighteen fish of each of four species were used, six fish for each state of rigor; the species were: white sucker (*Catostomus commersoni*) sauger (*Stizostedion canadense*), northern pike (*Esox lucius*), and rainbow trout (*Salmo gairdneri*). In addition, six lake whitefish (*Coregonus clupeaformis*) and six yellow walleye (*Stizostedion vitreum*) were obtained from commercial sources; all were of unknown history and were in post-rigor state; they were filleted and used as above; parts of these same fillets were also used for

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assessing the effect of treatment on taste. Finally, 64 1-lb packages of frozen whitefish and yellow walleye fillets were secured from a local plant; half of each lot had been treated with a polyphosphate solution before freezing. These were used for consumer-type testing of effect of polyphosphate on taste.

Excepting the 64 frozen samples referred to above, all samples were handled according to the following procedure: immediately after filleting, each fillet was weighed and placed inside a polyethylene bag, or was weighed, then dipped for 2 min into a solution of 8% sodium tripolyphosphate (food grade, Griffith's Laboratories) containing 2% sodium chloride, drained on a stainless steel wire screen for 2 min, weighed again and placed inside a polyethylene bag. All bags were sealed with a wire twist band, transferred to a plate freezer at –30 C for 2 hr and stored at –26 for 2 weeks. Thaw drip was determined by placing a sample of known weight inside a large glass funnel resting on top of a graduated cylinder and letting it drip overnight for 16 hr at 13 C, and then weighing it again at the end of the drip period. Each unit used for drip determination was enclosed in a polyethylene bag to prevent loss of moisture. The Freshwater Fish Marketing Corporation was planning automatic treatment in its new plant; for automatic applications, Gordon (1971) recommended a 7.5–10.0% TPP solution containing 2% NaCl. For this reason an 8% TPP solution containing 2% NaCl was chosen for the present work; the concentration was thought to be high enough to warrant expectation of some drip control and sufficiently low to avoid adverse taste effects.

Taste tests were carried out by six experienced tasters; each taster passed judgment on each of six fish, comparing a treated sample with a control sample. Only three fish per session were used so that tasters assesssed no more than six samples at any one session. The portions were prepared by cutting the frozen samples into six approximately equal pieces, wrapping each piece in aluminum foil, and cooking over steam for 20 min. Another taste test was carried out by a consumer-type panel. Thirty-two staff members were selected at random, each was supplied with two one pound packages of one of the species, one package containing treated fillets, the other untreated fillets; they were asked to take the samples home, cook them according to their own recipes and report differences and preferences.

Results and discussion — In control samples there was no consistent pattern from one species to another between state of rigor and amount of drip released (Table 1). Dyer and Fraser (1961) and Sutton (1969) reported that maximum drip occurs in pre-rigor cod; Dyer and Fraser (1961) attributed it to rough handling before freezing; in the present investigation only rainbow trout showed maximum drip in pre-rigor samples. Individual variations in white sucker ranged from 3.5 to 10.2%; in all other species the spread was never greater than 3.5%. The thaw drip of the other five species is much lower than that of flounder (14%, Tarr and Sunderland 1940), halibut (6.1–15%, Young 1941), cod (4.3–12.8%, Chalker et al. 1965), and plaice (9.5–27.5%, Templeman and Andrews 1956).

As in the control samples, the pattern between state of rigor and amount of drip in treated samples varied from one species to another (Table 1). Only with white sucker and sauger was the pattern the same as that obtained with the control samples. Treatment reduced drip considerably in all species; the overall average for treated samples was 2.0% versus 5.0% for control samples, a reduction of 60%. Best thaw drip reduction was achieved with white sucker (average 70%); least drip reduction

TABLE 1. Thaw drip in control samples (C) in percent of initial weight and in samples treated with sodium tripolyphosphate (T) in percent of weight after treatment. Each value is the average of six fish; ranges in parentheses.

Species		Pre-rigor	In-rigor	Post-rigor	Overall average (18 fish)
White sucker	C	6.3(3.5–8.7)	8.3(6.0–10.2)	6.6(5.6–8.5)	7.0(3.5–10.2)
	T	1.5(1.4–2.4)	2.6(1.7–3.5)	2.0(1.3–2.8)	2.0(1.3–3.5)
Sauger	C	3.5(2.9–4.4)	5.2(4.5–6.4)	4.7(3.7–5.6)	4.4(2.9–6.4)
	T	1.5(1.2–2.4)	1.9(1.5–2.5)	1.7(1.3–2.1)	1.7(1.2–2.5)
Northern pike	C	4.9(4.7–5.1)	4.9(4.2–5.7)	6.5(5.1–7.7)	5.4(4.2–7.7)
	T	2.6(2.2–3.4)	2.0(1.8–2.3)	2.5(2.1–3.1)	2.3(1.8–3.4)
Rainbow trout	C	5.8(4.5–7.3)	5.2(4.0–5.9)	4.2(3.9–5.1)	5.0(3.9–7.3)
	T	2.1(1.4–2.3)	2.4(1.6–2.9)	1.7(1.3–2.0)	2.1(1.3–2.9)
Yellow walleye	C	–	–	3.2(2.7–3.5)	–
	T	–	–	1.4(1.0–2.1)	–
Whitefish	C	–	–	4.0(2.9–5.7)	–
	T	–	–	1.3(0.4–2.2)	–

occurred in pike, but it was still substantial at 57%. Because the natural thaw drip of freshwater fish is rather low when compared with marine species, treatment with TPP may be of questionable value in view of the increased production cost.

But apart from decreasing drip, treatment with TPP causes an immediate increase in weight due to absorption during dipping. J. H. Mahon obtained a patent (No. 3,036,923[U.S.], May 29, 1962) for controlling drip by means of phosphate dips; he reported weight pickups varying between 1.5 and 5.5%, In the present investigation, weight increase due to dipping ranged from 0.5 to 5.9% (see Table 2), minimum and maximum weight increase occurred in yellow walleye and whitefish, respectively. The weight increase due to dipping was usually equivalent to the weight lost on thawing so that treated products ended up after thawing weighing as much as before treatment; but the control samples weighed less than their original weight before

TABLE 2. Weight gain of fillets due to treatment with sodium tripolyphosphate in percent of initial weight. Each value is the average of six fish; ranges in parentheses.

Species	Pre-rigor	In-rigor	Post-rigor	Overall average (18 fish)
White sucker	3.0(2.5–3.4)	2.1(1.7–3.1)	2.2(1.8–2.6)	2.4(1.7–3.4)
Sauger	1.9(1.2–2.5)	2.4(2.0–2.9)	2.9(2.3–4.6)	2.4(1.2–4.6)
Northern pike	2.9(1.9–3.5)	2.7(2.5–3.0)	1.8(1.7–2.0)	2.4(1.7–3.5)
Rainbow trout	2.3(1.7–3.0)	2.6(2.3–3.3)	2.6(1.9–3.5)	2.5(1.7–3.5)
Yellow walleye	–	–	1.8(0.6–2.6)	–
Whitefish	–	–	4.1(2.4–5.9)	–

TABLE 3. Net average difference, in percent of initial weight, between treated and control fillets after thawing. Standard deviations in parentheses.

Species	Pre-rigor	In-rigor	Post-rigor	Overall average (18 fish)
White sucker	7.4(±1.2)	8.5(±1.3)	6.8(±1.1)	7.6(±1.4)
Sauger	3.8(±0.7)	5.7(±1.0)	6.2(±0.8)	5.3(±1.3)
Northern pike	4.7(±0.8)	5.4(±0.7)	6.0(±1.1)	5.4(±1.0)
Rainbow trout	5.9(±0.7)	5.5(±1.2)	5.3(±0.9)	5.5(±1.0)
Yellow walleye	–	–	3.5(±0.7)	–
Whitefish	–	–	5.4(±1.2)	–

TABLE 4. Effect of sodium tripolyphosphate on taste of yellow walleye and whitefish fillets.

Species	No. of fish	No. of judgments	Difference in taste		Preference when detecting a difference for		
			Yes	No	Control	Treated	Neither
Yellow walleye[a]	6	35	27(77%)	8(23%)	12(44.4%)	12(44.4%)	3(11.2%)
Whitefish[a]	6	36	31(86%)	5(14%)	14(45%)	11(42%)	6(13%)
Yellow walleye[b]	32	16	14(88%)	2(12%)	7(50%)	7(50%)	0
Whitefish[b]	32	16	9(56%)	7(44%)	5(56%)	4(44%)	0

[a]Assessed by six experienced tasters.
[b]Assessed by consumer-type panel.

freezing by an amount equal to the natural drip loss. Thus the net difference between the two lots was equivalent to the natural drip, a result which corroborates that of Dyer et al. (1964). Table 3 shows the net difference between control and treated fish after thawing; except for yellow walleye, the net difference is in excess of 5%. The highest savings was with white sucker (7.6%) and the lowest was with yellow walleye (3.5%). The 5% net gain achieved with five of the six species could no doubt serve as a basis for calculating the economics related to treatment of these species with TPP. Under plant conditions, treatment with TPP might lead to different results; pilot plant testing under actual operating conditions would be required to determine optimum treatment conditions.

The results of taste panel tests are listed in Table 4. The majority (about 80%) of experienced tasters detected a difference in taste between the control samples and the treated samples of both yellow walleye and whitefish, and preference was almost equally divided between control and treated samples. In the consumer-type panel, the majority of tasters (88%) detected a difference between treated and untreated yellow walleye samples, but only 56% could detect a difference between treated and untreated whitefish samples; with walleye preference was equally divided; with whitefish a slight preference was given to control samples.

The effects of TPP on taste should be determined on other species and possibly other treatment conditions should be sought in order to avoid the unpleasant soapy taste detected by some of the tasters.

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Macrophthalmia in the American Eel (*Anguilla rostrata*)

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VLADYKOV, V. D. 1973. Macrophthalmia in the American eel (*Anguilla rostrata*). J. Fish. Res. Board Can. 30: 689–693.

Three American eels (*Anguilla rostrata*) with unusually large eyes, obtained from the collections of the Museum of Zoology, University of Michigan (UMMZ 80616), are described. Two are males, 495 and 521 mm in total length, and the third, probably of the same sex, had an estimated length of 752 mm. The horizontal diameter of the eye was equal to the interorbital width in the smallest specimen, while in the two others it was greater. These fish, captured in 1926, came from a sample of elvers liberated into Sherman Lake, Michigan, about 35–40 years earlier.

VLADYKOV, V. D. 1973. Macrophthalmia in the American eel (*Anguilla rostrata*). J. Fish. Res. Board Can. 30: 689–693.

L'auteur décrit trois anguilles d'Amérique (*Anguilla rostrata*) provenant des collections du Museum of Zoology, Université du Michigan (UMMZ 80616), dont les yeux sont exceptionnellement grands. Deux sont des mâles, mesurant 495 et 521 mm de longueur totale, et le troisième, probablement aussi un mâle, a une longueur estimée de 752 mm. Le diamètre horizontal de l'oeil est égal à le largeur interorbitaire chez le plus petit spécimen, alors que chez les deux autres, il la dépasse. Ces poissons, capturés en 1926, proviennent d'un échantillon de civelles libérées dans le lac Sherman, Michigan, environ 35–40 ans auparavant.

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PETERSEN (1896), summarizing the changes in the European eel (*Anguilla anguilla*) from yellow to silver stage, noticed, among other features, the increase in size and weight of the eye of silver eels. In two males, both 14.5 inches in length, the yellow and silver stages had respective eyeball weights of 40 and 80 mg and diameters of 5 and 6 mm.

Several other authors such as Grassi and Calandruccio (1897), Schmidt (1906), Svärdson (1949), D'Ancona (1951), Rasmussen (1951), Schnakenbeck (1953), and Bertin (1951, 1956) also observed *A. anguilla* with large eyes in natural conditions. Many others (Boucher et al. 1934; Fontaine 1936; Tuzet and Fontaine 1937; Fontaine et al. 1964; and Boëtius and Boëtius 1967a) found that injection of hormones resulted not only in enlargement of gonads in the European eel, but also in macrophthalmia.

No striking enlargement of the eye of the American eel (*Anguilla rostrata*) in natural conditions has previously been observed (Vladykov 1955, 1964, and 1966). Therefore, the find of three American eels with unusually large eyes, true macrophthalmous specimens, is of significant interest.

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Materials and Methods

Eel specimens were preserved, usually in 4–5% formalin, and kept in collections from a few months to 40 years.

To demonstrate changes in the size of eyes of adult American eels, 893 specimens were selected (Table 1). Only the horizontal diameter, being somewhat longer than the vertical diameter, was studied.

All measurements and weights were made on formalin preserved specimens. In Table 2 the body proportions, designated as a–b, a–c, a–d, and a–e, were measured according to Ege (1939, p. 7).

The other measurements were as follows: *i*, least bony interorbital width; O, horizontal diameter of the eye; *p*O, preorbital distance (or snout length) from the front margin of the orbit to the anterior tip of the upper jaw; and P, length of pectoral fin.

All measurements were made typically on the left side of the fish. Total length was taken from the tip of the lower jaw, mouth closed, to the posterior end of the middle caudal rays.

Anguilla rostrata with Large Eyes from Sherman Lake, Michigan

In the collections of the Museum of Zoology, University of Michigan, was present a sample

UMMZ 80616 consisting of a head (sex ?) and two entire male specimens of *A. rostrata* whose respective lengths were 495 mm and 521 mm (Fig. 1). These eels were collected by L. Sager around October 1, 1926, in Sherman Lake, Kalamazoo County, Michigan. Eels were first introduced in Sherman Lake in 1887 (R. M. Bailey personal communication) and from 1887 to 1891 nearly 2 million elvers were planted in Sherman Lake and other landlocked water of Michigan (Anon. 1893).

The three eels with unusually large eyes originated from one of the above-mentioned plantings.

TABLE 1. The horizontal eye diameter (O) in percentages of the interorbital width (*i*) in the American eel (*Anguilla rostrata*) of both sexes.

Length-class (*mm*)	Sex	No. specimens	O/*i* percentages							Avg.	Range
			40–49	50–59	60–69	70–79	80–89	90–99	100		
200–299	♂	11	–	–	4	2	5	–	–	74.0	62.5–83.3
	♀	19	–	–	2	8	6	3	–	78.1	66.7–90.0
300–399	♂	27	–	–	3	12	7	4	1	79.9	61.5–100.0
	♀	169	–	10	62	61	33	3	–	72.8	52.9–91.7
400–499	♂	4	–	–	–	–	3	–	1	88.9	81.3–100.0
	♀	295	2	24	105	100	53	11	–	71.2	45.5–93.6
500–599	♀	201	6	55	82	46	11	1	–	64.4	42.9–93.3
600–699	♀	50	3	18	22	5	1	1	–	61.6	46.7–93.8
700–799	♀	14	–	8	3	2	1	–	–	60.7	50.0–80.0
800–899	♀	41	5	26	6	4	–	–	–	56.0	46.7–75.0
900–999	♀	36	14	21	–	1	–	–	–	50.8	40.0–70.6
1000–1099[a]	♀	22	*9*	*11*	2	–	–	–	–	*50.0*	*45.5–60.9*
1100–1199	♀	4	–	4	–	–	–	–	–	54.5	52.0–59.1
Total for males		42	–	–	7	14	15	4	2	79.2	61.5–100.0
Total for females		851	39	177	284	227	105	19	–	67.1	40.0–93.8

[a]A female of the length-class 1000–1099 mm with unusually small eyes (38.5%) is not included in the table.

TABLE 2. Comparison between the largest *Anguilla rostrata* males from Canada and specimens with unusually large eyes from Sherman Lake, Michigan. (* indicates estimated values)

	Canada (Quebec)			Sherman Lake, Michigan		
	♂	♂	♂	♂	♂	?
Total length in mm	400	405	419	495	521	752*
Horizontal diameter of eye (O) in mm	8.0	6.5	6.5	13.0	15.0	19.0
In % of total length						
Head length (*a–e*)	12.0	12.6	12.4	12.7	12.3	12.5*
Predorsal distance (*a–b*)	30.3	33.0	32.7	32.3	32.2	32.3*
Preanal distance (*a–c*)	40.8	41.7	41.1	42.0	41.8	41.9*
Length of pectoral fin (*P*)	5.0	4.2	4.8	7.5	7.5	6.4*
Interorbital width (*i*)	2.0	1.9	1.9	2.6	2.5	2.3*
Horizontal diameter of eye (O)	2.0	1.6	1.6	2.6	2.9	2.5*
Preorbital distance (*p*O)	1.9	2.1	2.1	2.7	2.3	2.1*
In % of head length						
Interorbital width (*i*)	16.7	14.7	15.4	20.6	20.3	18.1
Horizontal diameter of eye (O)	16.7	12.7	12.5	20.6	23.4	20.2
Preorbital distance (*p*O)	15.6	16.7	17.3	21.4	18.8	17.0
In % of interorbital width						
Horizontal diameter of eye (O)	100.0	86.7	81.3	100.0	115.4	111.8
In % of preorbital distance						
Horizontal diameter of eye (O)	*106.7*	*76.5*	72.2	*96.3*	*125.0*	*118.8*

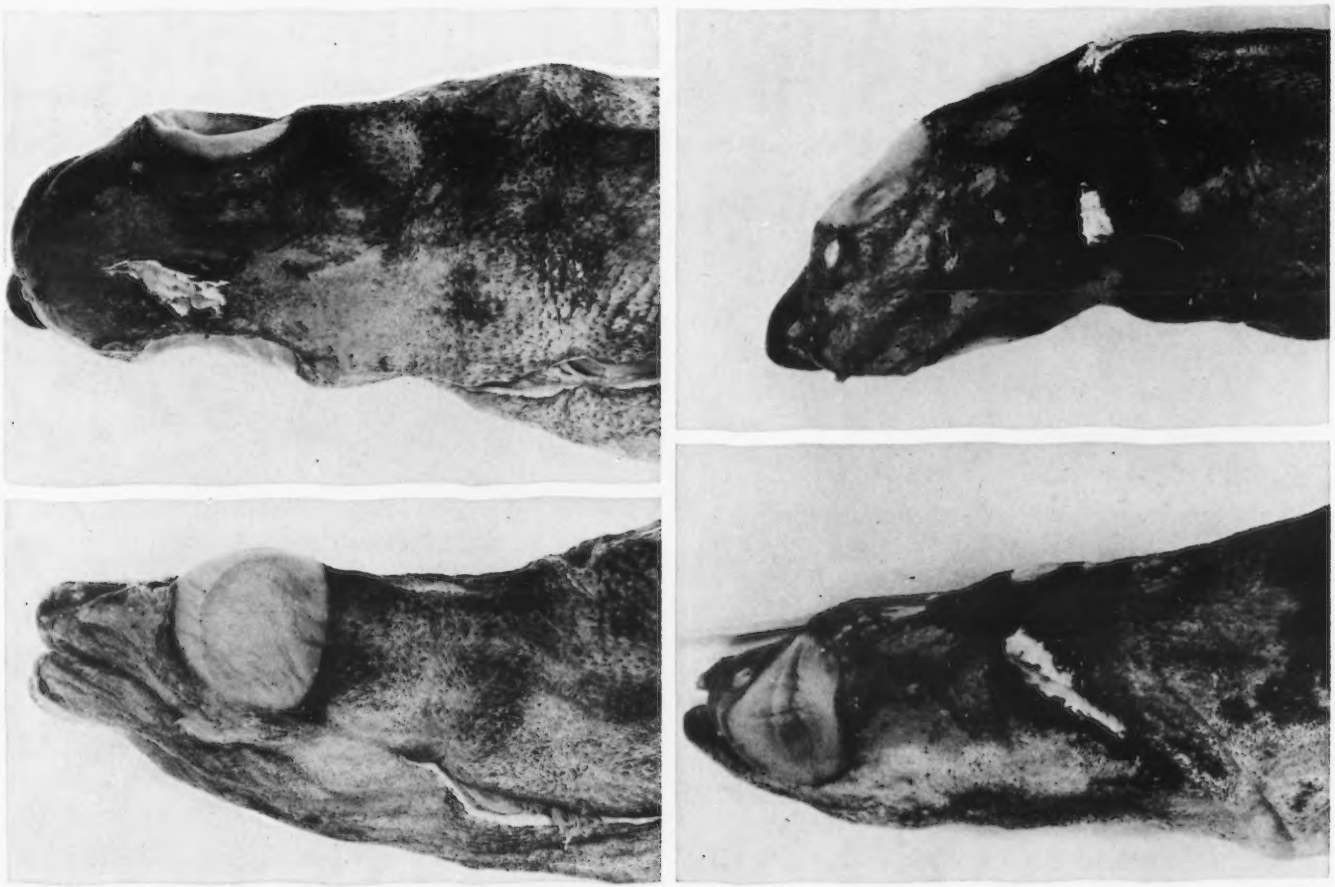

FIG. 1. Macrophthalmous American eel (*Anguilla rostrata*) from Sherman Lake, Michigan. (*Left*) Specimen of unknown sex; O/*i* = 112%, estimated length 752 mm. (*Right*) Immature male; O/*i* = 115%, length 521 mm.

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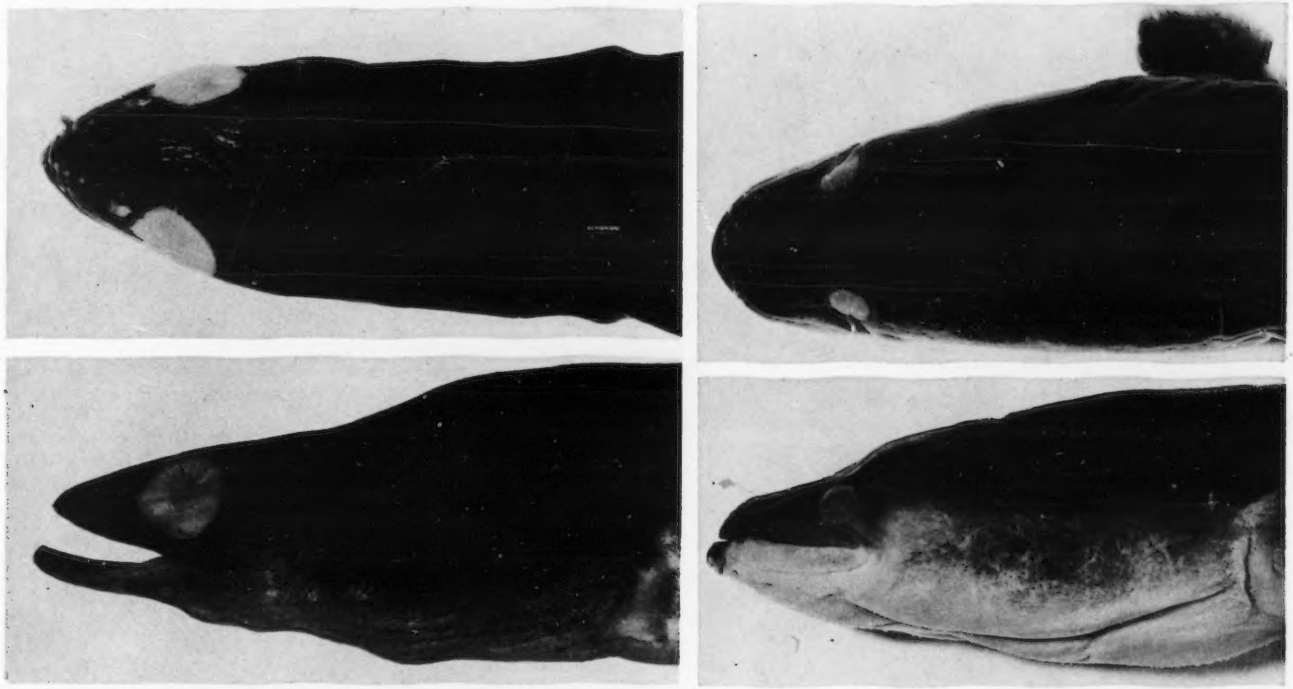

FIG. 2. (*Left*) Immature *A. rostrata* male, 345 mm in length, from Quebec, with large eyes (O/*i* = 100%). (*Right*) Immature *A. rostrata* female, 502 mm in length, from Shark River, Trinidad, with small eyes (O/*i* = 59%).

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Body proportions — Due to preservation in a small jar, the specimens were badly bent but were straightened as much as possible; details of body proportions are given in Table 2.

In comparison with male specimens from Canada, the three Sherman Lake eels differ particularly in interorbital width, preorbital distance, and horizontal diameter of the eye expressed as a percentage of head length. In addition, the horizontal diameter of the eye in the Sherman Lake specimens not only differed as a percentage of the interorbital width but also in absolute values.

The black pectoral fins in the Sherman Lake specimens were pointed instead of rounded as in typical specimens, and their length expressed in percentage of the total length was 6.4–7.5% against only 4.2–5.0% in Canadian specimens.

Two male specimens from Sherman Lake measured 495 mm and 521 mm, respectively, in total length. This was an unusually great size. Although it is not known to what sex the head (Fig. 1, *left*) belonged, it was unusually long, equal to 94 mm against 63 and 64 mm for the two males. Assuming that the relation between the head length and the total length in all three Sherman Lake specimens was the same, then the total length of the largest specimen would be equal to 752 mm. If it was a male, then its length was unusual. If it was a female, then the diameter of its eye was unusually great, equal to 111.8% of the interorbital width (Fig. 1, *left*).

Stage of maturity — Although the eyes of the two Sherman Lake males were unusually large, their gonads were little developed. The maximum width of the testes was only 5 mm in the smallest specimen but their appearance was normal. In the 521-mm specimen the testes were also about 5 mm in width, but were severely deformed. The stage of maturity of these specimens would be about 3. Spawning specimens belong to stage 5. The definition of stages of maturity of eels is comparable to that of *Salvelinus fontinalis* (Vladykov 1956).

Age — If the specimens under discussion originated from the earliest planting in Sherman Lake, then their age would be nearly 40 years. If, however, they originated from the last planting of 1891, then their age would be 35 years. In either case, the eels were much older than American eels normally found in nature.

Comparison between Eels from Different Areas

Size — Although *A. rostrata* females can reach up to 1275 mm in length and 6.2 kg in weight (Vladykov 1955; 1966), the males are much smaller. Among 171 males collected from Trinidad to Quebec and reported by Vladykov (1966), the largest specimen measured 375 mm. Boëtius and Boëtius (1967b) obtained three male American eels from Bermuda ranging from 295 to 327 mm. In Table 1, 42 males are mentioned, nine of which were collected in Trinidad and Florida, with a maximum size of 375 mm, while the remaining 33 were obtained in Canada from 1968 to 1971. The largest Canadian specimen was 419 mm in length (Table 2). Therefore, the two Sherman Lake males of 495 and 521 mm in length are real giants, and are similar to the maximum size of 510 mm reported for male European eels, *A. anguilla* (D'Ancona 1960).

Maturity and horizontal development of the eye — All Canadian specimens, even the largest one, from 345 mm to 419 mm, and even those with large eyes the diameter of which was equal to the interorbital space, were immature fish in the stage of maturity 0 or 1.

The Sherman Lake males were in a more advanced stage of maturity equal to about 3. Such a small development of the testes is rather surprising because the eels were unusually large and old. The fact that these fish stayed continuously in fresh water should not be considered detrimental to gonad development. In the case of *A. anguilla*, the maturation of males kept in fresh water was possible by hormone administration (Olivereau 1961).

Among 42 males, collected from Trinidad to Quebec, only two fish had an eye diameter equal to the interorbital space, both were from Quebec waters. One was 345 mm long with an eye diameter of 7.5 mm (Fig. 2, *left*) and the other was 400 mm long with an eye diameter of 8 mm. In general, among the 42 males, the horizontal diameter of the eye expressed in percentages of the interorbital width varied from 61.5% to 100.0% with an average of 79.2% (Table 1). This diameter for Sherman Lake males varied from 100.0% to 115.4%.

A. rostrata females, even those taken during their seaward migration, have smaller eyes than the males. Among 851 females, the eye diameter in interorbital space varied from 40.0% to 93.8% with an average of 67.1%. The most frequent occurrence of eye diameter for males was 70–90% and, in females, 60–80% (Fig. 2, *right*).

Blackford (1899) reported the capture of a female eel in an advanced stage of maturity on May 8, 1898, in the sea at Atlantic Highlands in relatively shallow water, between 2 and 3 fathoms. This eel was 420 mm in length and weighed 135 g. Its eggs were 0.4 mm in diameter and were "readily shaken free from the ovarian tissue." This was the most

mature *A. rostrata* female known. According to Blackford, its eye was not "notably larger than in similar specimens from the same locality."

Boëtius and Boëtius (1967a) concluded their observations on hormone treated male European eels by saying that the "eye-size increases continuously from the immature Syrski-stage (I) until a maximum is reached in stage 4–5 which corresponds to the process of spawning. During the postspawning stages (5–7) the eye-size decreases."

In spite of the general belief that enlargement in the eye-size is a good indication of advanced stage of maturity in European eels, it is not commonly supported by observations in nature. Only two *A. anguilla* males have been described as being macrophthalmous and possessing greatly developed testes. One, 340 mm in length with an eye diameter of 10 mm, from Denmark was observed by Schmidt (1906, p. 238). The second, 335 mm in length from Hamburg Bay, was described by Schnakenbeck (1953). All other males observed by several authors (Petersen 1896; Grassi and Calandruccio 1897; Svärdson 1949) had large eyes but their testes were not well developed. Similar observations on females with large eyes (Svärdson 1949; Rasmussen 1951; D'Ancona 1951) showed that their ovaries were far from being mature.

Observations on the Sherman Lake eels should be interpreted in the same sense as Svärdson (1949) did for the European eel. He believes that there are several stages through which even silvery eels should pass to obtain the nuptial dress. According to him, "in this process the enlargement of the eye comes at a comparatively early stage." This agrees very well with our observations on the Sherman Lake specimens in which macrophthalmia did not correspond to an advanced stage in gonad maturation, although the possibility that the gonads have secondarily regressed cannot be completely dismissed. It supports once more the idea that the enlargement of the eye diameter precedes maturation. The increase in eye-size is observed in both sexes of *A. rostrata*; however, in males the diameter of the eye is proportionally larger than in females (Table 1).

Age — The life span of American eels varies with the sex, the females living longer. The oldest female was taken in the St. Lawrence River in October 1971 near Kamouraska, Quebec, and was 21 years old. It measured 1125 mm in length and weighed 4891 g. The oldest male was that taken in Crecy Lake, New Brunswick, in June 1967. It measured 389 mm, weighed 101 g, and was 14 years old. The Sherman Lake eels were thus older than any known female and their age was more than double that of the males taken in natural conditions.

Although the Sherman Lake eels were no doubt very old, their age estimate was not as great as that mentioned for *A. anguilla* by Svärdson (1949). He reported the interesting story of "Putte," the Swedish eel celebrity. It was caught in 1863 as an elver at the age of three, and kept in captivity until it died in the autumn of 1948. Many owners inherited it and it finished its days at the age of 88 years in the Museum of Hälsingborg.

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A Simple Method for Recording Fish Heart and Operculum Beats Without the Use of Implanted Electrodes

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ROMMEL, S. A., JR. 1973. A simple method for recording fish heart and operculum beats without the use of implanted electrodes. J. Fish. Res. Board Can. 30: 693–694.

Recording fish heart and operculum beats directly from the water is possible within a limited range of water resistivity. This method can minimize fish handling in some physiological experiments.

ROMMEL, S. A., JR. 1973. A simple method for recording fish heart and operculum beats without the use of implanted electrodes. J. Fish. Res. Board Can. 30: 693–694.

L'enrégistrement direct, dans l'eau, des battements cardiaques et operculaires des poissons est possible sur une gamme étroite de résistivité de l'eau. Cette méthode peut minimiser la manipulation des poissons au cours d'essais physiologiques.

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ELECTROCARDIOGRAMS are used in many physiological and behavioral experiments. One drawback of this technique is the trauma induced in handling fish. This report describes a method by which cardiac and opercular beats can be recorded from a gently restrained, freely swimming fish without implanted electrodes. Goodman and Weinberger (1971) demonstrated the feasibility of this method with the salamander (*Nectalurus* sp.), using an array of electrodes. More recently Spoor and Drummond (1972) described a similar method for recording general body activity of aquatic animals in gradient tanks.

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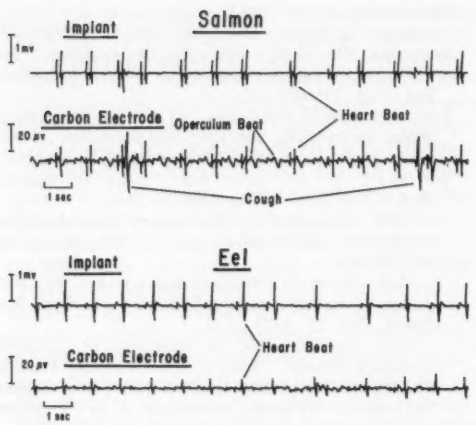

FIG. 1. Selected recordings of heart and operculum beats. The upper tracing is from an Atlantic salmon (*Salmo salar*) with a body length of 20 cm, and the lower tracing is from an American eel (*Anguilla rostrata*) with a body length of 50 cm. "Implant" refers to the recordings obtained from surgically implanted electrodes. "Carbon electrode" refers to the recordings from the same fish at the same time, but from the carbon electrodes.

Materials and methods — Soft plastic mesh cages placed in plexiglass aquaria were used to restrain the fish so that the long axis of its body was usually aligned in one direction; there was sufficient room for the fish to turn around. Maximum electric signals were obtained from electrodes fastened to either end of the mesh cages. Electrodes consisted of carbon rods with leads to a Grass Model 7 Polygraph. Low frequency swimming movements and high frequency electrical noises were eliminated with electronic filtering; low filter at 10 Hz, high filter at 35 Hz.

Atlantic salmon (*Salmo salar*), 15–24-cm standard length (to end of caudal pecuncle), and American eels (*Anguilla rostrata*), 35–56-cm standard length, were used. The cage sizes were 35 × 9 × 20 cm for salmon and 60 × 9 × 20 cm for eels.

Fish were tested in water resistivities from 4000 ohm-cm to 40 ohm-cm (0.25 milli-mho/cm to 25 milli-mho/cm, respectively). The method was usable in waters with resistivity between 4000 ohm-cm and 400 ohm-cm, but was not reliable in 40 ohm-cm water. Best results were obtained in water of approximately 1000 ohm-cm.

Results — As a check on the recordings, electrodes were implanted in several fish by the method described by McCleave et al. (1971). Occasionally a salmon coughed, as shown in Fig. 1, presumably to clear its gills (Saunders 1961). Selected results from salmon and eels are given (Fig. 1). Excellent agreement between implanted electrode recordings and carbon rod water electrodes was obtained. In some cases (not shown in figure) the operculum and heart beat amplitudes were approximately the same magnitude. In such cases changing the low frequency filter to 1 Hz permitted indentification of operculum beat.

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Some Simple Methods of Estimating Mortality Rates of Exploited Fish Populations

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SSENTONGO, G. W., AND P. A. LARKIN. 1973. Some simple methods of estimating mortality rates of exploited fish populations. J. Fish. Res. Board Can. 30: 695–698.

Some simple equations are derived from mortality functions that enable estimation of the total mortality coefficient from the mean age and the age of first capture, or the mean length and the length of first capture, of fish in a catch.

SSENTONGO, G. W., AND P. A. LARKIN. 1973. Some simple methods of estimating mortality rates of exploited fish populations. J. Fish. Res. Board Can. 30: 695–698.

A partir de fonctions mortalités, nous avons établi quelques équations simples, qui permettent d'estimer le coefficient de mortalité totale à partir de l'âge moyen et de l'âge à la première capture, ou à partir de la longueur moyenne et de la longueur à la première capture.

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WHERE detailed information based on growth and tagging studies on fish is not available, it is useful to consider the relation between mean age and the age of first capture, or of mean length to the length of first capture, as indicators of the total mortality coefficient. As rates of exploitation increase, the mean age and length are evidently closer to the age and length of first capture. This note derives the appropriate relations from the usual mortality function where recruitment is continuous and where recruitment takes place at discrete intervals.

Mean age and mortality rate — The conventional representation of mortality in an exploited fish population is

$$N_t = Re^{-Z(t-t_c)} \tag{1}$$

where N_t is the number at age t, R is the recruitment at age of first capture, and Z is the total mortality coefficient.

Where recruitment is continuous, the probability density function of the random variable t is given by

$$P(t) = Ze^{-Z(t-t_c)} \qquad \text{for } t > t_c. \tag{2}$$

Hence, as indicated by Beverton and Holt (1956), in a random sample catch, where the mean age, $\bar{t}$, is given by

$$\bar{t} = \frac{1}{n}\sum_{i=1}^{n} t_i$$

the expected mean age, $E(t)$, is

$$E(t) = \bar{t} = \frac{1}{Z} + t_c \tag{3}$$

from which $$\hat{Z} = \frac{1}{\bar{t}-t_c}. \tag{4}$$

Equation (4) gives a biased estimate when based on random samples from natural populations for which Z is not known. The equivalent unbiased estimator (except for $n = 1$) is

$$\hat{Z} = \frac{1}{\bar{t}-t_c}\cdot\frac{n}{n+1} \tag{5}$$

with the variance of Z given by

$$\text{Var}(\hat{Z}) = \left(\frac{n}{n+1}\right)^2 \cdot \frac{1}{n(\bar{t}-t_c)^2}. \tag{6}$$

Expression (5) enables estimation of the total mortality coefficient from samples for which the mean age and the age of first capture can be measured. Table 1, for example, gives estimates of Z and S^2 (Z) for *Tilapia esculenta* of Lake Victoria (Uganda) corresponding to various mean ages of capture (data for t_c from Garrod 1963).

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For fish populations with discrete age-groups, the number of fish at age $t+1$ is related to the number of fish at age t by

$$N_{t+1} = N_t \cdot e^{-Z}$$

so that

$$\frac{N_{t+1}}{N_t} = e^{-Z}. \tag{7}$$

The total population (N) with constant annual recruitment is

$$N = N_0 + N_0e^{-Z} + N_0e^{-2Z} + \ldots + N_0e^{-\infty Z}. \tag{8}$$

When the age of first capture is t_c, and if all fish above age t_c are equally likely to be caught, the probability of obtaining a fish of age t is

$$P(t) = (1 - e^{-Z})\, e^{-Zt} \qquad \text{for } t > 0. \tag{9}$$

The expected value of the average age ($\bar{t}$) in a sample catch is given by

$$E(t) = \bar{t} = \frac{e^{-Z}}{1-e^{-Z}} + t_c \tag{10}$$

which, with rearrangement, yields the estimate of the mortality coefficient

$$\hat{Z} = ln\left(\frac{\bar{t}-t_c + 1}{\bar{t}-t_c}\right). \tag{11}$$

Again, this estimate is biased. The unbiased estimator is

$$\hat{Z} = ln\left(\frac{\bar{t}-t_c + 1}{\bar{t}-t_c} \cdot \frac{n}{n+1}\right) \tag{12}$$

for which the unbiased variance estimate is

$$\text{Var}\,(\hat{Z}) = \left(\frac{n}{n+1}\right)^2 \cdot \frac{1}{n} \cdot Z^2. \tag{13}$$

Expression (12) enables estimation of the mortality coefficient when recruitment is discrete (say at yearly intervals) and where mean age of first capture is known. Table 1 gives estimates of Z and S^2 for *T. esculenta* for the discrete recruitment situation. For similar mean ages, estimates of the mortality coefficient are lower for discrete recruitment.

TABLE 1. Estimates of total mortality rate and its variance, based on mean age in catch ($\bar{t}$) and the age of first capture ($t_c = 2.5$ years, Garrod 1963) for *Tilapia esculenta* in Lake Victoria for various sample sizes (n), when recruitment is assumed to be continuous and when recruitment is assumed to be at discrete intervals.

	n = 500		n = 1000		n = 1500		n = 2000	
$\bar{t}$	Z	S^2 (Z)	Z	S^2 (Z)	Z	S^2 (Z)	Z	S^2 (Z)
				Recruitment continuous				
3.0	1.99	.00796	1.99	.00399	1.99	.00266	1.99	.00199
3.5	0.99	.00199	0.99	.00099	0.99	.00066	0.99	.00049
4.0	0.66	.00088	0.66	.00044	0.66	.00029	0.66	.00022
4.5	0.49	.00049	0.49	.00024	0.49	.00016	0.49	.00012
5.0	0.39	.00031	0.39	.00015	0.39	.00010	0.39	.00007
5.5	0.33	.00022	0.33	.00011	0.33	.00007	0.33	.00005
6.0	0.28	.00016	0.28	.00008	0.28	.00005	0.28	.00004
6.5	0.24	.00012	0.24	.00006	0.24	.00004	0.24	.00003
7.0	0.22	.00009	0.22	.00004	0.22	.00003	0.22	.00002
				Recruitment discrete				
3.0	1.09	.00239	1.09	.00120	1.09	.00080	1.09	.00060
3.5	.69	.00095	.69	.00047	.69	.00031	.69	.00023
4.0	.50	.00051	.50	.00025	.51	.00017	.51	.00013
4.5	.40	.00032	.40	.00016	.40	.00010	.40	.00008
5.0	.33	.00022	.33	.00011	.33	.00007	.33	.00005
5.5	.28	.00016	.28	.00008	.28	.00005	.28	.00004
6.0	.25	.00012	.25	.00006	.25	.00004	.25	.00003
6.5	.22	.00009	.22	.00004	.22	.00003	.22	.00002
7.0	.19	.00007	.19	.00004	.20	.00002	.20	.00002

Mean length and mortality rate — Because it may be difficult to determine the age of tropical fish, it is useful to consider expressions in which age is not an explicit variable. The von Bertalanffy equation

$$l_t = L_\infty\ (1-e^{-k(t-t_o)}) \tag{14}$$

is widely accepted as descriptive of growth in fishes. In practice, length at a given age is variable, but the distribution may not be symmetrical, and the variability may not be the same for various ages. For simplification, it will be assumed that l_t is fixed for age t.

Expression (14) may be rearranged to express time as a function of length

$$t = \frac{1}{k}\left(-ln\ (1 - \frac{l_t}{L_\infty})\right) + t_o. \tag{15}$$

The quantities y and y_c may be defined, respectively, as

$$y = -ln\left(1 - \frac{l_t}{L_\infty}\right)$$

and

$$y_c = -ln\left(1 - \frac{l_c}{L_\infty}\right)$$

where l_c is the length of first capture.

By replacement in expression (15)

$$t - t_o = \frac{1}{K}\cdot y$$

and

$$t_c - t_o = \frac{1}{K}\cdot y_c.$$

Replacing t and t_c in equation (1) by $\frac{1}{K}\cdot y$ and $\frac{1}{K}\cdot y_c$, respectively, gives

$$\frac{N_t}{R} = e^{-\frac{Z}{K}\cdot(y-y_c)}. \tag{16}$$

The probability density function of the random variable y is given by

$$P(y) = \frac{Z}{K}\ e^{-\frac{Z}{K}\cdot(y-y_c)} \tag{17}$$

from which an unbiased estimate of the ratio Z/K may be derived as

$$(\hat{Z/K}) = \frac{n}{n+1}\cdot\frac{1}{\bar{y} - y_c} \tag{18}$$

where $\bar{y}$ is the mean of y values calculated from knowledge of the mean length ($\bar{l}_t$) in various samples of individual fish.

The unbiased estimator for the variance of Z/K is

$$\text{Var}\ (\hat{Z/K}) = \left(\frac{n}{n+1}\right)^2\cdot\frac{1}{n(\bar{y} - y_c)^2}. \tag{19}$$

Estimation of Z can be made if K is known. Knowledge of K implies knowledge of age for the usual techniques of fitting the von Bertalanffy equation. However, K may be estimated from tagging data without explicit knowledge of age. Then Z would be given by

$$\hat{Z} = K\cdot\left(\frac{n}{n+1}\right)\cdot\left(\frac{1}{\bar{y} - y_c}\right). \tag{20}$$

Table 2 gives estimates of Z based on equation (20) for *T. esculenta* in Lake Victoria in the north

TABLE 2. Estimates of total mortality rate and its variance, based on mean length in catch (l_t) and the length of first capture (l_c = 22 cm), for various sample sizes (n) of *Tilapia esculenta* in Lake Victoria. Recruitment is assumed to be continuous. The values of the parameters of the von Bertalanffy growth equation are: K = 0.32 and L_∞ = 33.8 cm.

	n = 500		n = 1000		n = 1500		n = 2000	
$\bar{l}_t$	Z	S^2 (Z)	Z	S^2 (Z)	Z	S^2 (Z)	Z	S^2 (Z)
23	3.60	.02590	3.60	.01300	3.61	.00868	3.61	.00651
24	1.71	.00589	1.72	.00295	1.72	.00197	1.72	.00148
25	1.08	.00236	1.08	.00118	1.09	.00079	1.09	.00058
26	0.77	.00118	0.77	.00059	0.77	.00039	0.77	.00029
27	0.57	.00066	0.57	.00033	0.58	.00022	0.58	.00016
28	0.44	.00040	0.45	.00020	0.45	.00013	0.45	.00010
29	0.35	.00025	0.35	.00012	0.35	.00008	0.35	.00006
30	0.28	.00015	0.28	.00007	0.28	.00005	0.28	.00003
31	0.22	.00009	0.22	.00004	0.22	.00003	0.22	.00002
32	0.16	.00005	0.17	.00002	0.17	.00001	0.17	.00001

Buvuma area, estimates which agree closely with estimates of Z based on age data. For example, if, in a sample of 500, mean age of catch is 3 years, the estimate of Z is 1.99 (Table 1). A 3-year-old fish is 24 cm long. With a mean length in the catch of 24 cm and the length of first capture, l_c, as 22 cm, the ratio Z/K is estimated as 5.37; and, taking K as 0.32, Z is estimated as 1.71.

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Some Short-term Indicators of Sublethal Effects of Copper on Brook Trout, *Salvelinus fontinalis*

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DRUMMOND, R. A., W. A. SPOOR, AND G. F. OLSON. 1973. Some short-term indicators of sublethal effects of copper on brook trout, *Salvelinus fontinalis*. J. Fish. Res. Board Can. 30: 698–701.

Changes in cough frequency, locomotor activity, and feeding behavior of yearling brook trout appeared within 2–24 hr at copper concentrations as low as 6–15 μg/liter. Each of these responses appears to be useful for predicting the concentration range of copper likely to have no long-term effects on the species.

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Après exposition de 2 à 24 h à des concentrations de cuivre aussi faibles que 6–15 μg/litre, il se produit des changements de fréquence de toux, d'activité locomotrice et de comportement alimentaire. Chacune de ces réponses peut être utilisée, semble-t-il, pour prédire la gamme de concentrations de cuivre qui, vraisemblablement, n'aura pas d'effets à long terme sur l'omble de fontaine.

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THE purpose of this study was to evaluate changes in cough frequency, locomotor activity, and feeding behavior as possible short-term indicators of the long-term effects of copper on brook trout. Though long-term tests of a toxicant at all stages of the life history of a species are likely to give the most reliable information about the safe concentration, such studies require so much time that there is need for short-term methods for predicting the effects of chronic exposure (Mount and Stephan 1969; McKim et al. 1970). Among the requirements for evaluating a short-term test is a long-term study with which it can be compared. McKim and Benoit (1971) conducted such a study on the effects of copper on survival, reproduction, and growth of brook trout, and concluded that 9.5 μgCu(II)/liter was a safe concentration for this species in Lake Superior water. With their work as a basis for evaluation and using the same species and water supply, we describe the responses to short-term exposure to copper at concentrations near 9.5 μg/liter.

Materials and methods — Brook trout were studied in acrylic plastic electrode chambers (Spoor et al. 1971) measuring 24 × 11 × 14 cm, large enough to permit a 135–180-mm fish to move around in 2.6 liters of continuously flowing water. The chambers were insulated and the upper surfaces of the lids were painted to shield the fish from outside visual disturbance. Respiratory and other movements of the fish changed the potential difference between the electrodes; these changes were recorded on a polygraph. Before the series of experiments

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was started, the effects of coughing, locomotor activity, and feeding on the record were ascertained by activating an event marker while the fish were being observed directly. The coughs appeared as regularly recurring breaks in the ventilating rhythm. Locomotor activity caused marked irregularities on the record, together with temporary changes in ventilating frequency. Since feeding entailed vigorous movement, it was recorded as locomotor activity also, but could be recognized by the proximity with which it followed the introduction of food.

The water was drawn from Lake Superior, charcoal filtered, and then aerated in reservoirs before being delivered to the electrode chambers at 190 ± 5 ml/min at a temperature of 8.5 ± 0.5 C. The dissolved oxygen concentration remained close to saturation. The water had a total hardness (as $CaCO_3$) of 44–46 mg/liter; a *p*H range of 7.54–7.75; and a copper concentration of 0.7–1.4 μg/liter during the study period. Lighting was regulated by a time clock and incandescent light-dimmer control (Drummond and Dawson 1970); the daily photoperiod lasted for 12 hr starting at 6:45 AM.

Stock solutions of copper were prepared with reagent-grade cupric sulfate and distilled water. They were delivered to one or more of eight reservoirs by either a Mariotte bottle or a peristaltic pump and mixed well with lake water by aeration. Each reservoir supplied water to four electrode chambers. A composite 200-ml sample was collected from each group of the four chambers in which the trout were being exposed to a given concentration of copper. All samples were acidified and analyzed for total copper using the method described by Arthur and Leonard (1970). Each test concentration varied by not more than ±1 μg/liter from the scheduled nominal value.

Yearling brook trout were obtained either from the Cedar Island Trout Hatchery in Wisconsin, or from a stock (originally from the same hatchery) hatched and reared in the National Water Quality Laboratory. They averaged 157 mm (range 134–180) in total length and 39 g (range 20–56) in weight. All fish were maintained in large holding tanks at 8–10 C for several weeks before they were used in the experiments. They fed well in the holding tanks but would not feed for the first 3–5 days after transfer to the electrode chambers. No test was started until the fish were accepting food. The fish were fed a maintenance diet before and during tests except during those on locomotor activity.

Test procedures — No fish was used more than once after being exposed to added copper. At least 2–4 fish remained untreated during each test to serve as controls.

The effect of copper (6–15 μg/liter) on cough frequency was studied by comparing the rate of coughing during a 24-hr exposure with that of the same fish during the 24-hr preceding exposure. In some cases the 24-hr records were continuous; in the others each fish was monitored for a 15-min period each hour by use of an automated system. The 24-hr periods were started at the same time of day, 11:00 AM, to minimize the possible effects of a diel cycle. Coughs were counted for a 5-min period each hour and recorded as coughs per minute. The significance of the differences between frequencies before and during exposure to the copper concentrations was tested by the paired *t*-test. Differences between groups of fish at different concentrations were tested by chi-square.

One group of four fish was exposed to 115 μg/liter (slightly above the 96-hr TLm reported by McKim and Benoit 1971), and another group of four was exposed to 55 μg/liter.

The effect of copper on locomotor activity was studied by comparing the amount of activity during the first 2 hr of exposure to copper (6–60 μg/liter) with the amount shown by the same fish the day before the test and with controls in unmodified lake water. Recording was conducted for several hours before the copper was introduced to ensure that the pretreatment activity was similar to that of the day before. The amount of activity was estimated by measuring periods of activity along the horizontal axis of the record, and expressed as percent of time active. This was done before the fish were fed, so that feeding behavior did not affect the recording. In addition, four fish were kept at 12 μg/liter for a week and four more were exposed to that concentration for a 2 hr day for a week to test the effects of continued or repeated exposure.

Feeding behavior was monitored at 2 and 24 hr after the flow of copper (6–24 μg/liter) had been started. To keep the data on feeding as objective as possible, we used as the primary indicator any departure from the normal aggressive feeding pattern. The percent of fish that fed aggressively was then calculated. In addition, eight fish were exposed to 12 μg/liter continuously for 6 days and four fish were exposed to the same concentration without interruption for 14 days to show whether they would start feeding aggressively again.

Results — (*Effects on cough frequency*) — The cough frequencies varied from less than 1 to more than 3 a minute before the trout were exposed to copper (Table 1). However, each fish maintained a relatively constant rate that seldom varied by more than 6–8% during 24 hr period, and the mean frequency changed but little from one day to the next. The fish varied considerably in their response to copper. The cough frequency of some fish increased markedly while that of others did not change. The variation among individuals is reflected in the ranges before and during treatment.

At 55 and 115 μg/liter the cough frequencies increased so rapidly that the effects could be seen clearly within a few minutes after the exposure had been started. The rates continued to increase at these concentrations until by the 20th hr they averaged 6.3 and 7.4 coughs a minute at 55 μg/liter and 115 μg/liter, respectively. After the 20th hr they began to decline. At 6–15 μg/liter (Table 1) the changes in frequency followed the general pattern seen at the higher concentrations, but the increases were much smaller and developed so slowly that the change was not measurable until after the 4th hr.

The mean cough frequencies tended to increase with copper concentration, even within the narrow range of 6–15 µg/liter, and at 9 µg/liter the differences from both the 6 and 0 µg/liter values were significant ($P<0.01$). The comparisons between frequencies before and during exposure were based on the 16-hr period between the 5th–20th hr because the differences were greater during this period than before or after it. The difference between frequencies at 12 and 9 µg/liter was also significant ($P<0.01$).

Effects on locomotor activity — On their first day in the electrode chambers the fish were active between 25–40% of the time. The activity subsided gradually, and by the time they had started feeding again they were seldom active more than 6% of the time. Most fish showed increased activity within minutes after the copper was introduced. The responses varied over a wide range at each concentration, but all groups were from 4 to 6 times as active as the controls. The percentages of time (means and standard deviations) that the trout were active during 2 hr of exposure to various nominal concentrations (µg/liter) of copper were:

Concentration	Mean±SD	No. of fish
0	4.1±2.6	16
6	15.7±14.6	13
9	25.1±17.0	22
12	17.3±14.1	36
15	22.8±21.4	16
26–60	21.8±16.7	20

The responses at 115 µg/liter were of the same order, as those at 6–60 µg/liter. The increased activity lasted for 6–8 hr at all concentrations, and then it subsided toward the pretreatment level. The activity of fish exposed to 12 µg/liter continuously or intermittently for a week returned to the pretreatment level by the end of the third day and remained at this level.

TABLE 1. Means and ranges of cough frequencies of brook trout for 5–20 hr on the day before exposure and for the same period during the day of exposure to five copper concentrations. **Difference between means at this concentration significant ($P < 0.01$).

Copper concentration (µg/liter)		No. of tests	No. of fish	Coughs/min	
Nominal	Range			Before exposure	During exposure
0	0.7–1.4	7	25	1.68(1.10–2.45)	1.72(1.00–3.30)[a]
6	6.0–6.5	3	11	1.51(1.05–1.85)	1.64(1.20–2.30)
9	9.0–10.0	5	19	1.75(1.05–3.93)	2.06(0.95–7.10)**
12	10.8–12.0	6	22	1.74(0.95–2.50)	2.20(0.90–3.20)**
15	14.0–15.0	2	8	1.61(1.05–2.40)	2.16(1.05–3.30)**

[a]Second day under control conditions; no copper added.

TABLE 2. Percentages of brook trout that fed during various periods of exposure to five nominal concentrations of copper.

Copper concentration (µg/liter)	Exposure period				
	2 hr	24 hr	2 days	6 days	14 days
6	61.5(13)[a]	100 (4)			
9	4.6(22)	16.7(6)			
12	5.6(36)	12.5(8)	25.0(8)[b]	87.5(8)[b]	100(4)[b]
15	6.3(16)	0.0(4)			
17–24	0.0(20)	0.0(16)			

[a]Number of fish.
[b]Feeding was sluggish, not of the normal aggressive type.

Effects on feeding response — Untreated fish fed aggressively and continuously until all food offered them had been consumed. Feeding was markedly less aggressive or ceased after 2 hr at copper concentrations of 9 µg/liter and above, and nearly 40% of the fish at 6 µg/liter did not feed (Table 2). The fish began to feed again in what appeared to be the normal manner within 24 hr at 6 µg/liter, but not at 9 µg/liter and above. Fish maintained continuously at 12 µg/liter resumed feeding gradually, but the response remained sluggish (Table 2).

Discussion — Because changes occurred in cough frequency, locomotor activity and feeding behavior at copper concentrations near the 9.5 µg/liter considered safe by McKim and Benoit (1971), each of these responses shows promise as a short-term predictor of the concentration range at which chronic exposure might have no permanent effect. Each has advantages and disadvantages, depending upon the type of information required.

Cough frequency was the best of the three indicators for showing stress according to the copper concentration, but the change developed more slowly than in locomotor activity. Increase in activity is a very sensitive indicator, if observations can be made within the first few hours of exposure. The speed with which the reaction appears is its most useful aspect. The change in feeding behavior lasts the longest, but the indicator is less objective than the other two.

Since the responses can be obtained with yearlings, another possible time-saving advantage lies in the fact that the tests can be conducted at any time of the year. Brook trout are the most sensitive to copper during their alevin-juvenile stage of development (McKim and Benoit 1971), which restricts direct testing to the time of year this stage is available.

Our results are consistent with those of others concerning trout. The initial increase in cough frequency, its gradual subsidence during continued exposure, and its relation to the copper concentration are similar to the sequence described by Walden et al. (1970) for the cough frequencies of rainbow trout in dilutions of kraft mill effluent. The initial peak in activity, and return to the original level, were like those described by Dandy (1967) for brook trout at copper concentrations of 0.1 and 1.0 mg/liter. The gradual resumption of feeding at low concentrations of copper may account for McKim and Benoit's (1971) finding that the growth of young brook trout was retarded for the first 23 weeks at copper concentrations of 9.5 µg/liter and lower.

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Effects of Cooking on Concentrations of PCB and DDT Compounds in Chinook (*Oncorhynchus tshawytscha*) and Coho (*O. kisutch*) Salmon from Lake Michigan[1]

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SMITH, W. E., K. FUNK, AND M. E. ZABIK. 1973. Effects of cooking on concentrations of PCB and DDT compounds in chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon from Lake Michigan. J. Fish. Res. Board Can. 30: 702–706.

Concentrations of polychlorinated biphenyls (PCBs) and DDT compounds in chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon steaks were reduced slightly by cooking. In chinook, poaching and baking caused reductions of PCBs and pesticides of 2–8% while baking in nylon cooking bags reduced residue levels 11–16%. No significant differences in residue levels were found between chinook steaks cooked with and without skin, while steaks from the anterior halves contained less PCB and pesticide on a fat basis than steaks from the posterior halves. Analyses of a limited number of coho steaks indicated that they were lower in PCB and DDT compounds than the chinook steaks but showed no consistent pattern of reduction due to cooking method.

SMITH, W. E., K. FUNK, AND M. E. ZABIK. 1973. Effects of cooking on concentrations of PCB and DDT compounds in chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon from Lake Michigan. J. Fish. Res. Board Can. 30: 702–706.

Les concentrations de biphényls polychlorés (PCB) et de résidus de DDT dans des steaks de saumons chinook (*Oncorhynchus tshawytscha*) et coho (*Oncorhynchus kisutch*) ont été réduites légèrement par la cuisson. La cuisson du saumon chinook à la vapeur ou au four entraîne une réduction des PCB et pesticides de 2 à 8%, tandis que la cuisson au four dans des pochettes de nylon réduit la concentration de résidus de 11 à 16%. Nous n'avons décelé aucune différences significatives de concentration de résidus entre des steaks de saumon chinook cuits avec ou sans la peau. Par ailleurs, des steaks prélevés sur la moitié antérieure du poisson contiennent moins de PCB et de pesticides sur base de lipides que ceux de la moitié postérieure. L'analyse d'un nombre limité de steaks de saumon coho révèle qu'ils contiennent moins de PCB et de composés de DDT que ceux de saumon chinook, mais ne démontrent pas de tendance régulière à une réduction par la cuisson.

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Two recent studies have investigated the effects of various methods of cooking for the removal of chlorinated hydrocarbon pesticides in lake trout and coho salmon (Wanderstock et al. 1971) and bloaters and perch (Reinert et al. 1972). No work has been reported on chinook salmon, however, nor have the residues attributable to PCBs (polychlorinated biphenyls) been reported. It is known that PCBs are present in the Great Lakes ecosystem in higher amounts than the DDT compounds (Henderson et al. 1971) and that they may also interfere in gas chromatographic analyses of pesticides, thus enhancing the apparent amounts of DDT and its analogs.

It was the purpose of this study, therefore, to investigate the effects of three cooking methods on the residue levels of the PCB and DDT compounds present in chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon from Lake Michigan.

Procedures — Five male chinook ($\bar{x}$ = 58 cm; 43.2 kg) as well as one male (49 cm; 20.9 kg) and one female (48 cm; 20.4 kg) coho salmon were collected from the Manistee River weir in Michigan as the fish were passing upstream from Lake Michigan to spawn. The salmon were wrapped in aluminum foil, packed immediately in ice, and, within 4 hr, frozen whole at –20 C. Two weeks later, 2.54-cm steaks were sliced from the frozen whole bodies. Steaks were individually wrapped, coded so that slices from all areas of the fish were used for raw analyses and for cooking by each of the three

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methods. These frozen slices were returned to the freezer. Prior to cooking, each steak was halved to provide samples for raw and cooked analyses.

Raw samples of skin and flesh taken from the anterior and posterior halves (before or after the dorsal fin, respectively) were analyzed separately for PCB and pesticide residues. Samples from the two ends were cooked with and without skin and each component as well as cooking drip were analyzed separately. Visible fat deposits along the lateral line and belly were removed and analyzed with the skin piece so that flesh samples contained only intramuscular fat.

Thawed and cleaned half steaks were cooked by 1) baking at 177 C, 2) poaching in boiling salt water (5% NaCl), and 3) baking at 177 C in cooking bags manufactured from clear nylon film. All samples were cooked to an internal temperature of 75 C, as recorded in the dorsal muscle with an iron constantan thermocouple lead from a potentiometer.

Hexane-acetone extraction, acetonitrile partitioning, and Florisil-Celite column cleanup for PCB and pesticide residues, as well as fat analyses, followed procedures outlined by Yadrick et al. (1971). Entire flesh samples were blended for 10 sec to achieve a homogeneous mixture before approximate 10-g duplicate samples of raw or cooked flesh were taken. Each entire skin piece and any adjacent adipose tissue was also minced and blended with dry ice before each sample was analyzed separately (Bennville and Lindle 1970). Poaching liquid was blended before two 30-ml aliquots were removed. Pans and the bags in which samples were baked were rinsed with a mixture of hexanes to insure inclusion of all drip. The entire drip sample was used for one analyses.

Gas chromatographic analyses were carried out with a Beckman GC 4 chromatograph equipped with a discharge electron capture detector. It was fitted with a 1.83-m × 3.5-mm stainless steel column packed with 4% DC-11 on Gas Chrom Q (60/80 mesh) and was operated at column, inlet, and detector temperatures of 175, 230, and 255 C, respectively. Helium flow rates used were 40 ml/min for the column and 120 ml/min for the discharge side of the detector. Standards of Aroclors[3] 1248 and 1254, *p,p'*-DDT[4], *p,p'*-DDD[5], and *p,p'*-DDE[5] were prepared using nanograde hexane.

Differentiation and determination of the particular chlorinated hydrocarbons in the samples were confirmed by consultation with the Pesticide Research Center, Michigan State University, and through thin-layer chromatography. It was determined that the substances were the PCBs designated as Aroclors 1248 and 1254 and the DDT compounds *p,p'*-DDE, *p,p'*-DDD, and *p,p'*-DDT. Because Aroclor 1254 interfered with gas chromatographic readings of DDT and its analogs with like retention times, a method of separating the substanesc was necessary. Therefore, standard curves of Aroclor 1254 were run to determine the heights of peaks that would occur at like retention times to DDE, DDD, and DDT, and the height of the peak that, in fish samples, appears independently. The ratios of those peaks that occur at like retention times to DDE, DDD, and DDT to the independently occurring peak of 1254 were determined. These values were 1.25, 0.81, and 0.44 for DDE, DDD, and DDT, respectively. Amounts of interference in each sample were determined by multiplying the height (millimeters) of the independent 1254 peak by the appropriate ratio. This value was then subtracted from the appropriate height in the sample. The limitations of this approach were recognized; however, the same techniques were applied to raw and cooked samples. Thus, relative changes due to cooking were determined. Percentages of fat were determined as outlined by Yadrick et al. (1971) and calculations of parts per million of PCB and pesticide residues were based on fat content.

Data were analyzed for variance attributable to species, cooking method, individual fish, whether flesh was cooked with skin or without, and the position in the body from which the sample was taken. Duncan's multiple range test (Duncan 1957) was used to pinpoint sources of significant differences.

Results and discussion — Percentages of fat in the flesh of chinook and coho salmon were much lower than values reported in the literature (Stansby 1956; Lovern 1934; Buhler et al. 1969). It should be noted, however, that the salmon in this study were on a spawning run and would be living on body fat. Coho salmon contained higher levels of fat than chinook salmon (Table 1).

Table 1 shows that small reductions occurred in PCB and pesticide residue levels from the raw to cooked state. The presence of PCBs and pesticides in the drip further indicates that cooking reduces residue levels. Lipid and residue compositions of drip samples were extremely variable with residue levels appearing disproportionately high in comparison to levels in fish due to variation in fat content as well as sample variation. When analyzed for variance, residue levels in flesh samples showed no significant differences attributable to cooking method. Analyses of drip samples indicated that the Aroclors 1248 and 1254 were significantly more ($P < 0.01$) concentrated in the fat of poaching liquid than in that from baked or baked-in-bag samples. Although the statistical comparisons showed no consistent pattern for residue removal due to cooking method, indications are that samples cooked by baking in nylon bags lost 11–16% of PCBs and pesticides while residues in samples cooked by baking or poaching were reduced by only 2–8%. The residue content of the individual fish varied considerably (Table 1). Also, chinook salmon consistently had higher levels of PCB and DDT compounds than did the coho salmon.

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[3]Monsanto Chemical Co., St. Louis.

[4]City Chemical Corp., New York; 99+% ESA reference standard.

[5]Pesticide Research Laboratory, Perrine, Florida; 98+% analytical standard.

TABLE 1. Averages[a] and (in parentheses) ranges for percent fat and parts per million (fat basis) of PCB and DDT compounds in raw and cooked chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon flesh.

Species and sample type	Sample weight	Fat	PCBs		DDT compounds		
			Aroclor 1248	Aroclor 1254	*p,p'*-DDE	*p,p'*-DDD	*p,p'*-DDT
				Raw			
Chinook	–[b]	2.65	18.17	273.03	41.20	4.24	23.94
		(0.99–3.9)	(9.0–53.2)	(125.6–792.4)	(20.3–103.1)	(0.0–9.7)	(5.5–74.5)
Coho	–[c]	3.59	14.35	155.41	27.74	3.25	14.57
		(2.2–5.2)	(5.5–21.2)	(107.5–240.5)	(15.0–37.6)	(1.0–6.8)	(9.0–23.8)
				Baked			
Chinook	89.3	4.00	15.40	274.34	34.32	5.69	25.37
Flesh	(56.9–124.4)	(1.6–8.0)	(6.4–54.6)	(106.5–1091.4)	(8.8–105.9)	(0.0–23.9)	(5.5–122.2)
	1.2	7.18	319.7	1968.6	185.9	28.2	76.3
Drip	(0.6–1.6)	(0.21–19.62)	(21.1–785.5)	(89.9–4422.2)	(16.5–542.8)	(0.0–78.79)	(0.0–457.0)
Coho	57.3	4.53	13.85	170.69	20.97	1.10	10.20
Flesh	(52.5–62.0)	(3.1–6.4)	(7.5–19.5)	(87.9–251.2)	(18.9–24.3)	(0.0–2.8)	(6.0–14.6)
	0.7	16.17	206.2	863.5	33.5	0.0	50.0
Drip	(0.4–1.0)	(1.56–44.69)	(29.8–352.5)	(135.6–1354.0)	(9.9–67.6)	(0.0–0.0)	(0.0–176.2)
				Poached			
Chinook	108.8	3.43	18.40	268.15	35.72	4.72	22.34
Flesh	(51.0–182.9)	(1.4–7.1)	(3.0–51.9)	(65.2–591.0)	(10.2–85.2)	(0.0–16.6)	(5.3–76.7)
	2105[d]	0.03	751.5	4095.2	103.6	67.2	156.3
Drip	(1983–2153)	(0.01–0.07)	(297.5–1190.0)	(1050.0–8805.0)	(0.0–433.0)	(0.0–310.0)	(0.0–450.0)
Coho	53.8	4.08	15.60	176.49	27.86	2.61	15.18
Flesh	(44.7–62.9)	(1.9–5.2)	(5.6–24.7)	(82.5–323.6)	(14.2–54.8)	(1.0–3.8)	(6.5–24.4)
	2156[d]	0.02	575.1	1893.0	21.3	27.5	75.1
Drip	(2127–2185)	(0.01–0.03)	(377.5–960.0)	(1296.0–3025.0)	(0.0–75.0)	(0.0–1100)	(0.0–232.5)
				Baked-in-Bag			
Chinook	103.4	3.61	13.77	223.55	32.24	5.11	22.06
Flesh	(71.2–167.8)	(1.0–6.0)	(5.7–44.1)	(85.8–542.7)	(10.4–71.5)	(0.0–17.7)	(1.7–73.0)
	17.8	0.89	242.0	1746.9	92.2	11.8	101.4
Drip	(8.9–26.9)	(0.02–3.58)	(59.3–475.0)	(217.7–5312.7)	(0.0–129.2)	(0.0–82.8)	(0.0–662.7)
Coho	62.5	3.96	13.48	165.77	24.95	13.11	14.95
Flesh	(48.4–76.6)	(2.9–5.4)	(12.1–15.2)	(101.2–191.3)	(20.5–27.2)	(1.9–3.8)	(9.3–17.5)
	8.3	6.28	96.2	484.0	64.7	0.0	34.2
Drip	(6.2–10.4)	(0.30–21.9)	(59.3–145.0)	(142.1–916.7)	(25.6–114.7)	(0.0–0.0)	(0.0–91.3)

[a]Chinook averages based on 10 raw and 20 cooked flesh samples each done in duplicate as well as 20 single drip analyses. Coho averages on 2, 4, and 4, respectively.

[b]Average raw weights for chinook that were baked were 133.0 (78.4–180.0), poached 128.0 (67.1–212.4), and baked in a bag 127.0 (83.4–198.9).

[c]Average raw weights for coho that were baked were 77.7 (69.9–85.4), poached 62.6 (53.6–71.5), and baked in a bag 72.5 (56.4–88.6).

[d]Remaining poaching liquid. 2325 g used originally.

Because salmon reportedly contain pesticides in higher amounts in fatty areas under the skin (Reinert et al. 1972; Dice 1969), the differences in residue content of steaks cooked with and without skin were investigated. Also, DDT concentrations are reportedly highest in some species of Lake Michigan fish in parts of the body with highest oil content (Reinert 1970). Therefore, the amount of PCBs and pesticides in the anterior and posterior halves of the chinook were examined (Table 2). In almost all instances where fat content was highest, in the anterior halves of both raw and cooked flesh of the chinook salmon, the PCB and DDT levels were lowest. Cooked anterior slices with significantly more fat ($P \leq 0.10$) had significantly less 1248 PCB and DDE. Values for other residues were also lower in the anterior slices but values were not significantly different. If the assumption that pesticide levels follow fat levels were true, then the highest residue levels would be expected in the high fat areas. Thus, the suggestion that cooking methods that leach fat are effective in removing pesticides (Dice 1969; Reinert et al. 1972; and Ritchey et al. 1969) is probably more applicable to fish with a high fat content.

The salmon used in this study contained a very low level of fat and only small amounts of residues were removed from the flesh itself where perhaps the PCB and DDT compounds would be more closely bound to the intermuscular fat. Even in raw flesh of chinook and coho steaks, percentage fat correlated negatively with levels of 1248 PCBs ($r = -0.734$; $P \leq 0.001$), 1254 PCBs ($r = -0.487$; $P \leq 0.01$), and *p, p'*-DDE ($r = -0.462$; $P \leq 0.01$). DDT values, however, exhibited no correlation with percentage fat in the raw flesh. This inverse relation could be related to the heavy demands on body fat for energy during spawning, which would deplete fat. Drip from samples cooked by the three methods showed no significant differences in the amounts of pesticide residues present between samples cooked with and without skin. This also indicates that PCBs and pesticides in the area under the skin, where leaching of fat would be facilitated, were not removed to any extent. Cooked skin samples showed no consistent pattern for changes in all PCB and pesticide residues among individual fish, cooking methods, or positions from which the samples were taken.

Conclusions — The cooking methods used generally caused a small decrease in the PCB and DDT compound residue levels with steaks baked in nylon bags containing lower levels than poached or baked steaks.

There was no apparent relation between the level of PCB or pesticide residues and concentration of fat in spawning chinook and coho salmon. PCBs and pesticide levels were higher where fat levels were lower. Steaks from the anterior halves of each fish contained less PCB and pesticide in their fat than steaks from the posterior halves, and

TABLE 2. Comparisons of averages[a] and (in parentheses) ranges for percentage fat and parts per million (fat basis) PCB and DDT compounds in chinook flesh cooked with skin and without, and between raw and cooked chinook flesh from the anterior and posterior halves.

Flesh state	Fat	PCBs		DDT compounds		
		Aroclor 1248	Aroclor 1254	*p,p'*-DDE	*p,p'*-DDD	*p,p'*-DDT
Cooked						
W/skin	3.73	14.65	242.26	32.49	4.61	20.77
	(1.4–8.0)	(6.1–51.9)	(95.4–556.9)	(10.0–76.1)	(0.0–14.8)	(1.7–73.0)
W/O skin	3.63	17.06	268.43	35.70	5.73	25.74
	(1.0–7.1)	(3.0–54.6)	(65.2–1091.4)	(8.8–105.9)	(0.0–23.9)	(5.3–122.2)
Raw						
Anterior	3.04	15.28	243.53	36.87	3.92	20.59
	(1.15–4.1)	(9.0–28.1)	(202.9–329.9)	(20.9–51.4)	(0.0–7.3)	(5.5–31.7)
Posterior	2.35	21.06	302.53	45.54	4.55	27.30
	(1.04–3.7)	(11.2–53.2)	(125.6–792.4)	(20.2–103.1)	(0.0–9.7)	(9.1–74.5)
Cooked						
Anterior	4.22	12.89	249.46	31.38	5.70	25.35
	(1.6–8.0)	(3.0–54.6)	(65.2–1091.4)	(8.8–105.9)	(0.0–23.9)	(1.8–122.2)
Posterior	3.14	18.82	261.23	36.81	4.65	21.17
	(1.0–5.6)	(6.1–51.9)	(106.5–591.0)	(10.4–85.2)	(0.0–16.9)	(1.7–76.7)

[a]Cooked and raw averages based on 30 end samples, respectively, analyzed in duplicate.

steaks cooked with skin contained less PCBs and pesticides than steaks cooked without skin. The differences were not significant, however.

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Electrophoresis of Fish Myofibrillar Proteins With Sodium-Dodecyl Sulfate-Polyacrylamide Gel[1]

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CHILDS, E. A. 1973. Electrophoresis of fish myofibrillar proteins with sodium-dodecyl sulfate-polyacrylamide gel. J. Fish. Res. Board Can. 30: 706–707.

A method for electrophoretic separation of fish myofibrillar proteins is described. After incubation in phosphate buffer containing β-mercaptoethanol, sodium-dodecyl sulfate (SDS), and urea, the proteins are separated by SDS-acrylamide gel electrophoresis.

CHILDS, E. A. 1973. Electrophoresis of fish myofibrillar proteins with sodium-dodecyl sulfate-polyacrylamide gel. J. Fish. Res. Board Can. 30: 706–707.

L'auteur décrit un procédé de séparation électrophorétique des protéines myofibrillaires de poisson. Après incubation dans un tampon phosphate contenant β-mercaptoéthanol, sodium-dodécyle sulfate (SDS) et urée, les protéines sont séparées par électrophorèse sur gel de SDS-acrylamide.

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TECHNIQUES for analysis of fish myofibrillar proteins are presently limited to myosin (Buttkus 1966) and actin and tropomyosin (Connell 1968). Techniques that would allow scrutiny not only of these proteins but also of actinins and inhibitory proteins would be most desirable. Recently Wilkinson et al. (1972) described a method for SDS-acrylamide gel electrophoresis of rabbit myofibrils. This report is on adaptaion of the technique for fish muscle myofibrils.

Materials and Methods — Whole fish myofibrils, from three species, were isolated by modification of a method described for mammalian muscle by Briskey and Fukaza-

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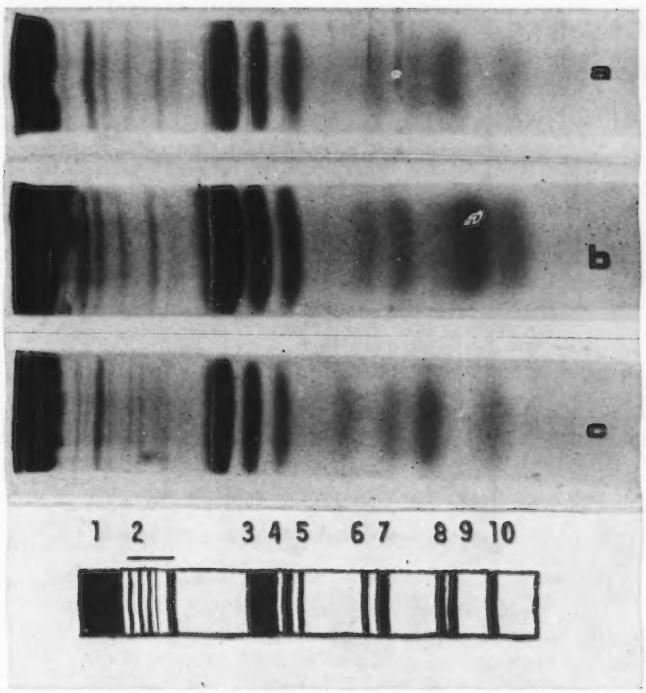

FIG. 1. Electrophoregrams of myofibrillar proteins of (a) ling cod, *Ophiodon elongatus*; (b) petrale sole, *Eopsetta jordani*; and (c) Pacific hake, *Merluccius productus*; and schematic (after Wilkinson et al. 1972) of rabbit. Schematic bands were suggested by Willkinson et al. (1972) to be (1) heavy chains of myosin, (2) actinins, (3) actin, (4) 37,400 MW component, (5) tropomyosin, (6) 25,600 MW myosin chain, (7) inhibitory factor, (8) Ca sensitizing factor, (9) 18,000 MW myosin chain, and (10) 15,000 MW myosin chain.

Childs — J. Fish. Res. Board Can.

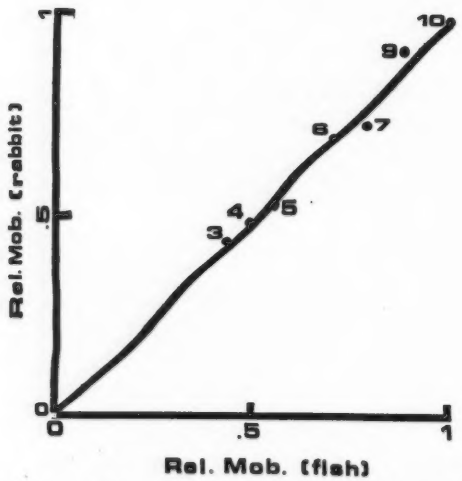

FIG. 2. Relative mobilities of most fish and rabbit myofibrillar protein bands during SDS-acrylamide gel electrophoresis. Each point represents the mean of five replicates. In no case was the standard error of the mean greater than the size of the point. See Fig. 1 for key to numbers.

wa (1971). All steps were performed at 0–4 C. Muscle from freshly killed fish was chilled in ice and minced. Twenty grams of the minced muscle was finely subdivided in a blender set at its lowest speed. The finely divided material was homogenized in the blender (at medium speed) for 1 min with five volumes of 0.025-M KCl, 0.039-M borate, and 5-mM disodium EDTA adjusted to *p*H 7.1 with KOH. The homogenate was centrifuged at 600 *g* for 15 min and the supernatant discarded. The pellet was resuspended in five volumes of the same buffer, and the suspension was homogenized in a blender (at medium speed) for 2 min and centrifuged at 600 *g* for 15 min. The supernatant was carefully decanted and discarded. The sediment (primarily a myofibril suspension) resting on top of the pellet was carefully decanted; diluted with five volumes of 0.1-M KCl, 0.039-M borate, and 5-mM disodium EDTA adjusted to *p*H 7.1 with KOH; and centrifuged at 400 *g* for 3 min. The supernatant was decanted and centrifuged at 600 *g* for 15 min, and the resulting supernatant was discarded. This pellet was repeatedly washed with a buffer of 0.1-M KCl, 0.039-M borate, and 5-mM disodium EDTA, as previously described (Briskey and Fukazawa 1971), to yield a purified myofibril preparation.

The myofibrils were prepared for electrophoresis by a modification of methods described for homogeneous protein preparations by Weber and Osborne (1969). All steps were carried out at room temperature. To solubilize the myofibrillar proteins, the myofibril sediment was suspended in 2 × its weight of 0.01-M sodium phosphate buffer at *p*H 7.0 containing 1% sodium dodecyl sulphate (SDS), 1% β-mercaptoethanol, and 8-M urea. The solubilized protein solution was dialyzed for 2 hr against 40 volumes of 0.01-M sodium phosphate buffer at *p*H 7.0 containing 0.1% SDS and 0.1% β-mercaptoethanol. For each protein sample, a mixture consisting of 0.03 ml of 0.05% bromophenol blue, 10 drops glycerol, 0.05-ml β-mercaptoethanol, and 0.50 ml of the dialysis buffer was prepared. A 0.25-ml portion of the dialyzed myobril solution was added to this mixture, and this served as the sample for electrophoresis.

SDS-acrylamide gels were prepared as described by Weber and Osborne (1969). The equipment and techniques used for electrophoresis were slight modifications of those originally described by Davis (1964). The reservoir buffer was prepared by diluting the gel buffer with two volumes of deionized, distilled water. The reservoir buffer was used only one time. Electrophoresis was carried out for 3 hr at a constant current of 8 mA/gel. Staining and destaining techniques were identical to those described by Davis (1964).

Utility of the Technique — Pacific hake (*Merluccius productus*), lingcod (*Ophiodon elongatus*), and petrale sole (*Eopsetta jordani*) electrophoregrams (Fig. 1) were each constant. Approximately 100 samples of each species were examined, and no deviations from the banding patterns shown in Fig. 1 were noted. To compare these data with those of rabbit myofibrillar proteins (Wilkinson et al. 1972), the relative mobilities (mobility of protein band/mobility of most mobile band) of all protein bands were calculated. When the relative mobilities of fish (x) and rabbit (y) myofibrillar proteins were plotted, there was a straight-line relation for most (Fig. 2) and only what may be the actinin group was skewed. Regression analysis of all other bands (except the Ca sensitizing factor, which could not be resolved from the 18,000 M.W. chain of myosin in rabbit and, according to Nockolds et al. (1972), may not occur in fish) provided a correlation coefficient of 0.982 and a regression equation given by:

$$y = 0.981\,(x) - 0.004.$$

From this statistical analysis, it is obvious that the two systems have a high degree of correlation. However, it should not be inferred that the proteins of the two systems are necessarily identical. Verification of the identities of fish myofibrillar proteins must await homogeneous isolation, characterization of biochemical activity, and amino acid analysis.

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A Comparison of Liquid Scintillation and Geiger–Müller Estimates of Primary Productivity in an in situ Experiment[1,2]

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WARD, F. J., AND M. NAKANISHI. 1973. A comparison of liquid scintillation and Geiger–Müller estimates of primary productivity in an in situ experiment. J. Fish. Res. Board Can. 30: 708–711.

For an in situ experiment conducted in Shiozu Bay, Lake Biwa, Japan, primary productivity estimates from liquid scintillation radioactivity counts of wet algae were generally higher than those from Geiger–Müller radioactivity counts of desiccated algae. Values at 0 m were similar, the G–M estimate at 0.5 m was 10% higher, but from 3 to 13 m the liquid scintillation values ranged from 11 to 33% higher than G–M estimates. The 20-m estimates were low and similar. Differences were caused primarily by ^{14}C losses during desiccation prior to G–M counting. Increasing loss rates between 0.5 and 3.0 m may have been caused by decreasing light intensity. On the basis of surface area, the estimate from liquid scintillation data was 27% greater than that obtained from G–M data.

WARD, F. J., AND M. NAKANISHI. 1973. A comparison of liquid scintillation and Geiger–Müller estimates of primary productivity in an in situ experiment. J. Fish. Res. Board Can. 30: 708–711.

Au cours d'un essai poursuivi in situ dans la baie Shiozu, lac Biwa, Japon, les mesures de radioactivité d'algues mouillées déterminée par scintillateur liquide étaient ordinairement plus élevées que celles obtenues sur des algues désséchées par compteur Geiger–Müller. A 0 m les valeurs sont semblables, à 0.5 m l'estimé G.–M. est 10% plus élevé, mais de 3 à 13 m, les valeurs des estimés par scintillement liquide sont de 11 à 33% plus élevées que celles obtenues par G.–M. Les estimés de 20 m sont bas et semblables dans les deux cas. Les différences sont dues surtout à des pertes de ^{14}C au cours de la dessication précédant le comptage G.–M. Une augmentation du taux de perte entre 0.5 et 3.0 m peut être due à une diminution d'intensité lumineuse. Sur une base de superficie, l'estimé par scintillement liquide est 27% plus élevé que celui obtenu par comptage G.–M.

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THE purpose of this paper is to compare primary productivity estimates based on radioactivity determinations made using liquid scintillation and gas flow Geiger–Müller techniques in an in situ experiment.

Wallen and Geen (1968), using liquid scintillation counting techniques, demonstrated that desiccation of algae on membrane filters could lead to losses of ^{14}C as great as 50% of "wet" values. In the laboratory, Ward and Nakanishi (1917) compared primary productivity calculated from liquid scintillation counting of "wet" filters with Geiger–Müller counts of desiccated ^{14}C labeled algae and found significant desiccation losses as well as an error associated with determining zero thickness activity. In a second laboratory experiment we determined that loss rate increased with decreasing light intensity during sample incubation (Nakanishi and Ward 1971). This result indicated that loss rate might increase with incubation depth in in situ experiments.

Materials and methods — Water samples were collected on November 27, 1970, from seven depths (0–20 m) in Shiozu Bay, Lake Biwa, Japan, using a plastic water sampler. Aliquots were placed in two "clear" and two

[1]Contribution No. 214 from the Otsu Hydrobiological Station, Kyoto University.
[2]Contribution from JIBP-PF No. 116.

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"dark" 100-ml BOD bottles for each depth. Each bottle was inoculated with approximately 2.4 μCi of ^{14}C-$NaHCO_3$. All bottles were then incubated for 4 hr (1030–1430) at the depths at which the sample had been collected. After incubation, 2 ml of 20% neutral formalin were added to each bottle.

From samples incubated at each depth two 40-ml aliquots from each "clear" bottle and a single 40-ml aliquot from one "dark" bottle were filtered through 0.45 μ Millipore HA membrane filters. Scintillation counting procedures and standardization of ampoules were described by Ward and Nakanishi (1971). Two 60-ml aliquots from each "clear" bottle and one or two 60-ml aliquots from "dark" bottles were filtered and fumed, but these filters and algae were attached to stainless steel planchets and desiccated over soda lime for approximately 1 week. The radioactivities of these desiccated filters were estimated using Geiger–Müller procedures (Ward and Nakanishi 1971).

The carbon dioxide carbon concentration in Lake Biwa water was 5.0-mg C/liter, determined by using an infra red CO_2 analyzer. Primary productivity was calculated from the mean radioactivities of paired "clear" bottles and paired and single "dark" bottle values using Strickland's (1960) formula. Specific and relative activities of added isotope were adjusted for aliquot volume. For scintillation data, specific activities of added ^{14}C and labeled algae were used rather than relative radioactivity estimates. The mean counting efficiency of the scintillation counter for all samples was 61%, with a range from 57 to 65%. Specific and relative estimates of the radioactivity of the ^{14}C stock ampoules were, respectively, 5,340,600 ± 52,000 dpm (0.95 confidence interval) and 3,610,000 cpm/ml.

Results — Basic radioactivity data (Table 1) are not directly comparable because scintillation data are estimates of specific activity (dpm) whereas G–M data are in terms of relative activity (cpm). Although replicates were too few to determine confidence intervals, agreement was generally good. Ranges expressed as percentages of mean counts ranged from 0 to 7.1% for scintillation "clear bottle" data and from 0 to 12.6% for G–M clear counts.

Productivity estimates obtained with the two radioactivity measuring instruments were relativety similar near the surface, but from 1.5 to 7.0 m the scintillation estimates were considerably higher (Fig.1). Below 7 m productivity was low and differences were minor.

The curve calculated from G–M radioactivity determinations had a clearly apparent peak at 0.5 m, whereas the comparable curve based on liquid scintillation count data had a much broader peak. Values at 0.5, 1.5, 3.0 m were similar.

Relative differences between estimates determined by the two counting methods increased rapidly with increasing depth below 0.5 m. The percentage differences, calculated as variations from Geiger–Müller data, at the various depths were:

Depth (*m*)	0	0.5	1.5	3.0	7.0	13.0	20.0
Difference (%)	7.1	−10.3	10.9	27.8	33.3	33.3	0

TABLE 1. Liquid scintillation and Geiger-Müller radioactivity data for the samples incubated at various depths in Shiozu Bay.

Depth (*m*)	Liquid scintillation (*dpm*)[a]		Geiger–Müller (*cpm*)[b]	
	Clear bottle	Dark bottle	Clear bottle	Dark bottle
0	6954	178	6382	209
	6938	–	6713	211
0.5	9653	367	10162	246
	9769	–	11526	241
1.5	8907	302	8602	184
	9495	–	7755	227
3.0	9015	230	6034	129
	9014	–	5801	–
7.0	2769	426	1701	195
	2944	–	1878	205
13.0	910	367	511	145
	887	–	510	–
20.0	773	531	321	101
	720	–	320	–

[a]From 40-ml samples.
[b]From 60-ml samples.

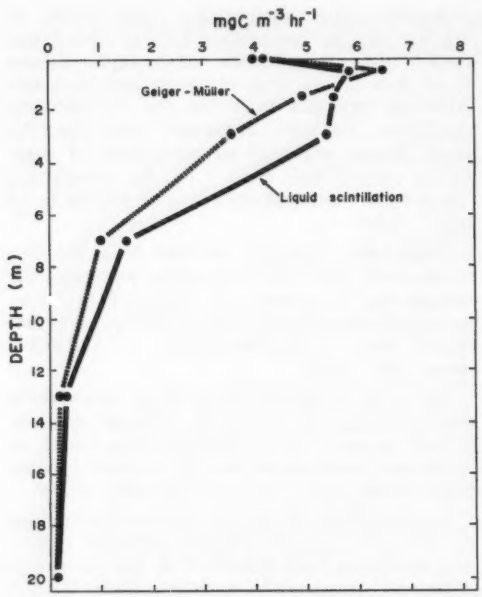

FIG. 1. Primary productivity at various depths in Shiozu Bay estimated from liquid scintillation and Geiger–Müller radioactivity determinations.

At 0.5 m the G–M value was higher than the liquid scintillation estimate.

Primary productivity per unit area was 38 mg-$Cm^{-2}hr^{-1}$ on the basis of the scintillation data and 30 mg$Cm^{-2}hr^{-1}$ for the G–M data, the former being 27% higher.

Discussion — The low estimates of primary productivity on the basis of G–M counts (Fig. 1) can be attributed to desiccation losses (Wallen and Geen 1968) and a constant error of about 11% associated with measuring the relative activity of introduced ^{14}C using the barium carbonate precipitate method (Ward and Nakanishi 1971).

In general, loss rate increased with increasing depth from the surface to 7 m. The values were similar and high at 7 m and 13 m but were 0 at 20 m, where primary productivity was negligible. The G–M estimate at 0.5 m (10% greater than the liquid scintillation value) cannot be readily explained. Logically, G–M values based on counts of similar samples should not be higher than liquid scintillation estimates.

During the experiment the factor showing the most variability from the surface to 20 m was light intensity, which decreased to 1% of the surface intensity at a depth of 13 m. Factors that might be associated with phytoplankton biomass and indirectly, therefore, with ^{14}C loss rate were relativety constant. For example, chlorophyll *a* estimates ranged from 1.14 to 1.28 mg/m^3 and particulate organic carbon values from 253 to 304 mg/m^3. Temperature, which might have independently affected photosynthesis and thus loss rate, was also constant (at about 13 C) from the surface to 20 m. Based on our previous work (Nakanishi and Ward 1971), decreases in light intensity with depth were probably the major cause of variations in loss rate although, as mentioned, a constant 11% could be associated with the G–M standardization procedure (Ward and Nakanishi 1971). Other factors involved may have been changing species composition with depth or changes in the physiological condition of the algae, but there was no evidence that these factors were associated with variations in loss rate.

The more rapid decrease between 0.5 and 3.0 m in the estimates based on G–M counts than in those based on liquid scintillation data may reflect an increasing accumulation of volatile substances within cells with increasing incubation depth and decreasing light intensity.

Desiccation losses are probably variable in in situ experiments, depending on incident solar radiation, light penetration, and, perhaps, algal biomass and productivity. At any rate, it seems unlikely that G–M data can be corrected by application of a simply derived coefficient. Results from this experiment and other recent work (Nalewajko and Lean 1972; Schindler et al. 1972) also lead to the conclusion that previously recommended methods for sample preparation and radioactivity determinations may cause primary productivity estimates from in situ data to be significantly in error. The range of errors resulting from desiccation of algae on filters occurring in natural systems has yet to be determined, but liquid scintillation counting procedures in conjunction with the use of dioxane fluors will eliminate them.

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Steam Distillation: A Simple Technique for Recovery of Petroleum Hydrocarbons from Tainted Fish

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ACKMAN, R. G., AND D. NOBLE. 1973. Steam distillation: a simple technique for recovery of petroleum hydrocarbons from tainted fish. J. Fish. Res. Board Can. 30: 711–714.

Simple steam distillation was found to be efficacious for isolation of hydrocarbons from contaminated whitefish. Gas–liquid chromatography on support-coated open-tubular Apiezon-L columns demonstrated that hydrocarbons recovered from the tainted fish had a composition pattern qualitatively similar in detail to that from a sample of diesel oil suspected as the source of contamination.

ACKMAN, R. G., AND D. NOBLE. 1973. Steam distillation: a simple technique for recovery of petroleum hydrocarbons from tainted fish. J. Fish. Res. Board Can. 30: 711–714.

Une simple distillation à la vapeur s'avère efficace pour l'isolement des hydrocarbures des poissons à chair blanche. La chromatographie en phase gazeuse-liquide sur colonne capillaire, genre tubulaire ouverte, avec support couvert d'Apiezon-L démontre que les hydrocarbures isolés de poissons infectés ont une composition qualitativement semblable, dans ses détails, à celle d'un échantillon d'huile à diesel soupçonnée d'être la source de contamination.

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WE have found that direct steam distillation of blends of fish muscle is very satisfactory for recovery of contaminating hydrocarbons of the diesel oil range, which would also include domestic fuel oils, both likely to be involved in product spillages and consequent problems with tainted fish.

Experimental — Samples — Fish caught through the ice of the Athabaska River in the spring of 1972 were judged unfit to eat by the fishermen. A probable point of origin for petroleum products entering the river appeared to be a discharge pipe below the Canadian National Railway yards in Jasper. Samples of diesel oil then in use in Jasper by Canadian National Railways and control samples of fish from a source ostensibly free of contamination were obtained before the tainted fish were examined.

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The Alberta fish examined consisted of five samples from the Athabaska River and a control group from Lake Minnewanka, Banff National Park, all caught in April 1972 (details in Table 1). Rainbow trout from Halifax Laboratory stocks served as methodology controls for groups 1–6, which were mostly whitefish.

Sample Processing — The fish were shipped to Halifax in polyethylene bags containing dry ice and stored at –40 C until ready for examination. Each lot was thawed and filleted when required. Approximately 15 g of fillet was blended with 100 ml of distilled water, transferred to a 250- or 500-ml round-bottom distillation flask, then the blender was rinsed with 50–100 ml of water, and the washings were added to the flask.

Gas–Liquid Chromatography on Apiezon-L — A support-coated open-tubular (SCOT) stainless steel column 0.02-inch internal diameter and 150 ft in length was employed in a Perkin-Elmer Model 990 gas–liquid chromatograph. The operation was isothermal at 180 C with

16 psig helium carrier gas. The flame ionization detector was operated with air at 500 ml/min and hydrogen at 40 ml/min. Each day after startup the helium pressure was raised to 60 psig for 30 min to clear any residual high-boiling components (septum bleed or previous day hydrocarbons). Following return to usual conditions, diesel oil solutions were analyzed daily to verify normal operation. Standard hydrocarbons in solution were run if required. Standard solutions were made of C_{10}–C_{15} aliphatic hydrocarbons singly or in combination in neohexane. Only glass-stoppered volumetric flasks were used. The experimental work on fish was carried out with neohexane as the volatile solvent and, again, only all-glass apparatus was used.

Steam Distillation — All steam distillations were carried out with 500- or 250-ml round-bottom flasks with standard taper necks. A gooseneck tube ran at an angle of approximately 30° for 150 mm to an integral vertical Liebig condenser. The inside of the condenser inner joint was loosely packed with fresh degreased coarse glass wool prior to each distillation from fish. All apparatus was rinsed with acetone and petroleum ether and dried shortly before use. When the apparatus was assembled all joints were wetted with water prior to commencement of heating. The joint and riser part of the condenser were wrapped in aluminum foil. Blends of fish muscle and water, or water for trials with authentic hydrocarbons or diesel oil in pure form or in neohexane solutions, were normally half the capacity of the flask. Antibumping granules were added and fish muscle blend was simmered for 30 min before distillation to reduce foaming, which carried particles of fish to the condenser tube. Such particles were, however, retained by the glass wool. Antifoam A (Dow Chemical Co.) reduced this foaming, but was discontinued because it caused a homologous series of peaks on GLC.

Condensate was collected to the 20-ml mark in a graduated 50-ml glass-stoppered centrifuge tube in ice, 2-ml neohexane was added, and the tube was shaken vigorously. In most samples the neohexane layer separated cleanly on standing for 1 hr or less in a refrigerator; in a few samples, the stable emulsion that formed was broken by addition of 50% KOH solution, 0.5 ml, followed by shaking and refrigeration overnight. The bulk of the neohexane extract, 1.6–1.8 ml, was transferred to a graduated 5-ml glass-stoppered centrifuge tube and the precise volume transferred recorded. Concentration to a volume appropriate for gas–liquid chromatographic analysis was effected with a gentle stream of nitrogen. As the first 20 ml of distillate provided a sufficient hydrocarbon sample, this approach was standardized for analytical work with fish.

All distillates were corun with a mixture of normal aliphatic standards (C_{12}–C_{17}) to identify the locations of these components. Unexpectedly, these alkanes were not as important as usually shown for diesel oil and similar types of volatile hydrocarbons (compare Fig. 1 with Jeltes 1969; Blumer et al. 1970; Connell 1971; Adlard 1972; Mackie et al. 1972) and were essentially absent from the control fish of both origins, despite their probable occurrence from biological sources in natural waters (Clark 1966; Oro et al. 1967; Blumer et al. 1970; Gelpi et al. 1970; Ruebelt 1970; Ackman 1971). The "C_{15}" material (Fig. 1) is a case of coincidence on Apiezon-L GLC of an unknown volatile material naturally present in fish, or derived as an artifact from the GLC column, as there is no reason for this alkane to be important in diesel oil or to occur in the control fish. Accordingly, this peak is labelled C_{15}-P to distinguish it from C_{15} alkane.

TABLE 1. Source and estimated diesel oil content in muscle of fish caught in Jasper National Park, Alberta, in 1972.

Sampling location and date	Sample Fish	Estimated diesel oil in fish muscle (*ppm*)
Athabaska River		
Group 1. Mile 12 Highway, Apr. 3 (R. Howarth)	A	29
	B	53
Group 2. Mile 12 Highway, Apr. 8	A	73
	B	61
Group 3. Mile 3 Highway, Apr. 8	A	80
	B	88
	B	83
Group 4. Old Fort Point, Apr. 9[a]	A	77
Group 5. Mile 12 Highway, Apr. 15	B	59
Lake Minnewanka, Banff National Park		
Group 6 (Control).	22	4 (max.)
	21	14 (max.)

[a]Another fish from this lot (from above the pollution source) had no obvious diesel oil hydrocarbon pattern.

Results and discussion — A preliminary study with diesel oil or authentic C_{10}–C_{18} hydrocarbons showed that approximately 25% of check samples were recovered in the first 20 ml of condensate, and 12–25% in a second 20-ml fraction. The compositions of both fractions collected were similar. Carbon tetrachloride extractions of the still residue from a diesel oil steam distillation showed essentially no volatile hydrocarbons, but tailing of the solvent peak was severe on Apiezon-L. Carbon disulphide as an extractant was more satisfactory for GLC as the solvent peak was negligible and confirmed that virtually no volatile hydrocarbons remained. It was found that the remainder of the hydrocarbons was held by surface effects in the vertical portion of the condenser and could be recovered

by tilting the condenser and rinsing with 20-ml water or with carbon disulphide. Combined recoveries of initial distillate and condenser holdup for tetradecane were 95%. For diesel oil, measured by total flame ionization detection response in the C_{12}–C_{15} range, recoveries were from 90 to 95%, and recordings of a few peaks with a long retention time suggested that correction for the content of these higher boiling hydrocarbons in the diesel oil reference solution would have improved comparison with the gravimetric standard. The mixture of authentic hydrocarbons did not change appreciably in chain-length composition, and the gas–liquid chromatographic pattern of the C_{11}–C_{15} range of the distillate diesel oil hydrocarbons (Fig. 1–I), useful for "fingerprint" comparisons, did not differ much from that of undistilled diesel oil (Fig. 1–II).

Most components from the reference diesel oil could be recognized in varying concentrations, but in distinctive peak groupings, in the steam distillates from Athabaska River fish (compare Fig. 1-I, 1-III, 1-V). As there are numerous likely aromatic or isomeric alkanes or both in diesel oil (Zitko and Carson 1970), somewhat different proportions of the various components might accumulate in various fish after fractionation by natural processes (Blumer et al. 1970). It is, however, unlikely that any peak would be completely eliminated (Cole 1971: Kawahara 1972). The qualitative presence of individual hydrocarbons in a complex mixture implicated as the origin of petroleum taint must be considered with some reservations as concentrations for threshold detections by man probably vary widely (Shipton et al. 1970).

Quantitation (Table 1) was based on averages for the peak heights for the lettered peaks A, D, and H, which were selected as reasonably clear of interference from adjacent components and were barely detectable in chromatograms of distillate from control fish, although peaks A and D may have been present in trace amounts (Fig. 1-IV). Normal alkanes were unsatisfactory as they could have originated from biological sources. Lake Minnewanka is frequented by motorboats that could contribute to the minor level of hydrocarbon content observed in control fish 21 and 22. Diesel oil levels (Table 1) were consistent and comparable with those for tainted fish from other studies (300 ppm in trout, Mackie et al. 1972; 24–300 ppm in mullet, Connell 1971). Levels in fish taken the same day 9 miles apart indicate exposure to the same basic oil (Fig. 1-III, 1-V).

Steam distillation has been used successfully to concentrate volatile hydrocarbons from diethyl ether extracts of fish muscle or blends of fish muscle (Vale et al. 1970; Connell 1971). It is simple and rapid and in our hands recovers nearly the total volatile portion of petroleum corresponding to diesel oil, without serious fractionation or disproportionation of components. This should, therefore, include one-quarter to one-third of most crude oils (Blumer and Sass 1972), as well as other fuel oil classes of materials, and should provide clean samples for study and identification by some of the applicable

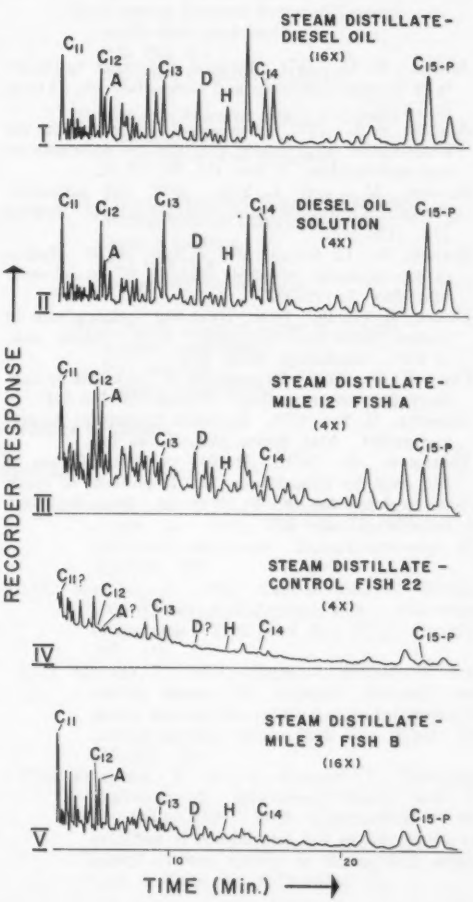

FIG. 1. Chromatograms comparing: I and II, steam distillate extract from 1-μliter diesel oil and starting material in solution; III and IV, steam distillates from tainted fish (Athabaska River, mile 12, below Jasper) and control fish from Lake Minnewanka (Banff National Park); III and V, steam distillates from tainted fish caught 9 miles apart in the Athabaska River on the same date. Attenuations (x) as marked, for GLC details see text.

physico-chemical techniques reviewed by Adlard (1972) and Kawahara (1972). Marine fish yield gas–liquid chromatographic blanks with minimal background peaks by a vaporization approach of this type (Deshimaru 1971a, 1971b).

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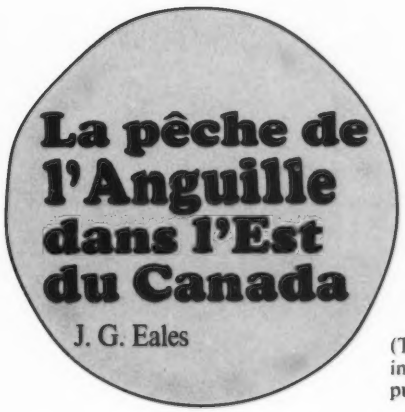

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